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Original Research

Circulating de novo lipogenesis fatty acids and all-cause mortality in a prospective Dutch population cohort

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Background: Circulating fatty acids (FA) from de novo lipogenesis (DNL) are associated with all-cause mortality in individuals with elevated CVD risk. However, compared to FA early in the DNL synthetic pathway, cis-vaccenic acid, one of the FA distal in the DNL synthetic pathway, has rarely been studied in a general population cohort. We hypothesized that circulating cis-vaccenic acid is more strongly related to all-cause mortality than other circulating DNL-related FA.

Objectives: The primary and secondary objectives of this study were to investigate the prospective associations of plasma levels of cis-vaccenic acid and other DNL-related FA with all-cause mortality in a general population, respectively.

Methods: We included 850 participants (mean ± SD age 53 ± 15 years) from the Dutch Lifelines cohort study. Circulating levels of palmitic (C16:0), palmitoleic (C16:1n7), cis-vaccenic (cis-C18:1n7), stearic (C18:0), oleic acid (C18:1n9) in plasma phospholipids (PL) and triglycerides (TG) were measured by gas chromatography. The associations of circulating cis-C18:1n7 and other DNL-related FA with all-cause mortality were assessed using Cox regression analyses.

Results: During a median follow-up of 9.3 (IQR: 5.4–10.8) years, 34 (4.0%) participants had died. In plasma PL, a 1-SD increase in cis-C18:1n7 was associated with an increased risk of all-cause mortality in univariate and multivariate models ($p<0.02$ for all), with a HR [95% CI] of 1.60 [1.13–2.25] after adjustment for age and sex.

Conclusions: Circulating plasma PL cis-C18:1n7 was associated with a higher risk for all-cause mortality. More studies are needed in different cohorts to verify and validate our results.

Introduction

Hepatic de novo lipogenesis (DNL) is a tightly regulated metabolic pathway in which excess dietary carbohy-

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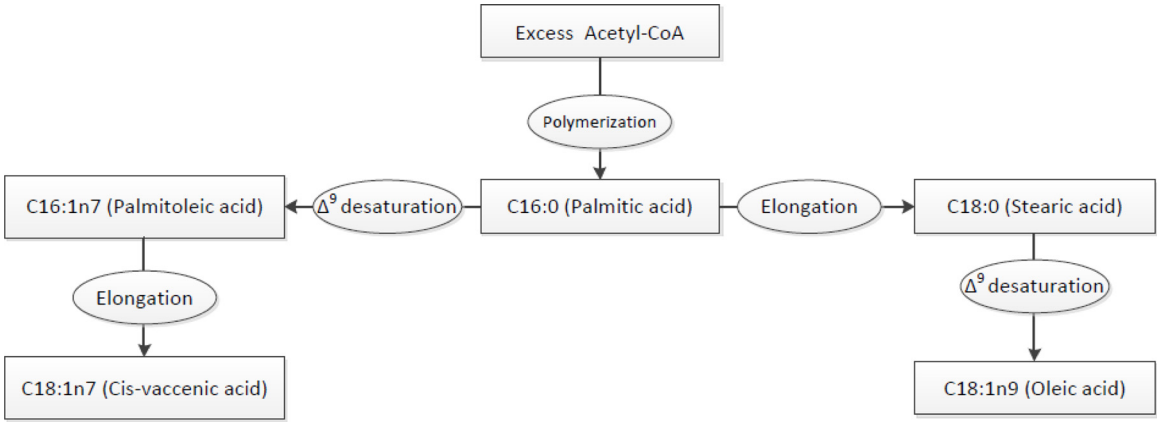

Fig. 1 The major saturated and monounsaturated fatty acids (FA) produced by the de novo lipogenesis (DNL) pathway, whereby acetyl-coenzyme A (acetyl-CoA) is polymerized to form fatty acids. The initial major FA of DNL is palmitic acid (C16:0), which can be processed by Δ^9 desaturation and/or elongation to palmitoleic acid (C16:1n7), cis-vaccenic acid (C18:1n7), stearic acid (C18:0), and oleic acid (C18:1n9).

drates, e.g., from starch, sugar, and alcohol, are endogenously converted into fatty acids (FA), which might eventually contribute to increased intrahepatic fat, insulin resistance, and dyslipidemia.[1,2] In line with this, DNL and circulating FA in DNL pathway have been increasingly linked to cardiometabolic diseases, such as type 2 diabetes (T2D), cardiovascular disease (CVD), and cancer.[2,3]

The major DNL-related FA are palmitic acid (C16:0) and stearic acid (C18:0), which can be desaturated through delta-9 desaturase to palmitoleic acid (C16:1n7) and oleic acid (C18:1n9), respectively. C16:1n7 can subsequently be elongated to cis-vaccenic acid (C18:1n7) (Fig. 1). Studies have shown that circulating C16:0, C16:1n7, C18:0, and C18:1n9 are associated with higher risks for T2D, CVD, and all-cause mortality.[2-6]

Circulating cis-C18:1n7 has rarely been related to clinical outcomes due to the methodological difficulties in separating cis- from trans-isomers. Recently, our team has developed a special assay to allow the separation of trans-C18:1n7 from cis-C18:1n7 in different plasma lipid compartments.[7,8] To our knowledge, circulating cis-C18:1n7 measured in plasma phospholipids (PL) have been associated with non-CVD mortality in a general population and a population with elevated CVD risk, respectively.[2,9] Circulating cis-C18:1n7 and other DNL-related FA from different plasma compartments and their association with clinical outcomes have seldomly been studied together.

In this study, we primarily investigated the association of circulating cis-C18:1n7 measured in plasma PL and TG with all-cause mortality in a general population-based prospective cohort study. In secondary analyses, we investigated the associations of circulating other DNL-related FA, i.e., C16:0, C16:1n7, C18:0, and C18:1n9 in plasma PL and TG, with all-cause mortality. Since cis-C18:1n7 is distal in DNL pathway and less abundantly present in the diet than the other distal FA (C18:1n9), circulating cis-C18:1n7 may depend more on DNL. Thus, we hypothesized that circulating cis-C18:1n7 would have a stronger association with all-cause mortality than circulating other FA in DNL pathway.

Methods

Study design and population

The Dutch Lifelines Cohort Study is a large prospective population-based cohort study and Biobank that examines the health and health-related behaviors of over 167,000 participants residing in the three northern provinces in The Netherlands. The first group of participants was recruited via their general practitioners. Then the recruited participants could indicate if their family members were also interested. Additionally, individuals interested in the study could also register through an online self-registration. Individuals with insufficient knowledge of the Dutch language, with severe psychiatric or physical illness, and those with limited life expectancy (<5 years) were excluded from the study. Participants (>18 years old) completed several questionnaires, including topics such as demographics, medication use, and diet. Participants were also invited to the Lifelines Research sites for a comprehensive biomedical assessment. All participants provided written informed consent before study entry. The Lifelines Cohort Study was conducted according to the principles of the Declaration of Helsinki and approved by the Medical ethical committee of the University Medical Center Groningen, The Netherlands (2007/152). A more detailed description of the Lifelines Cohort study and its representativeness can be found elsewhere.[10,11] For the current study, we used a subset of the baseline data, in which 864 participants were randomly selected from the Lifelines biobank. Participants with missing data on circulating FA or loss to follow-up at the endpoint were excluded before analysis, leaving 850 participants in this study (Supplementary Figure S1).

Study measurements

Anthropometric measurements and blood pressure (BP) were measured by well-trained staff. Anthropometric measurements were measured without shoes, in which body

weight was measured to 0.1 kg by the SECA 761 scale (Seca GmbH, Hamburg, Germany); height was measured to 0.5 cm using the Frankfort Plane position by the SECA 222 stadiometer (Seca GmbH, Hamburg, Germany).[11] BMI was calculated as body weight (kg) divided by height squared (m^2). Blood pressure was measured by Dynamap PRO 100V2 (GE Healthcare, Freiburg, Germany); systolic and diastolic blood pressures were measured 10 times within 10 min, and each of the average values of the last three readings was used as blood pressure parameters.[11]

Education, smoking status, and medication use were derived from self-administrated questionnaires. Education, as defined by the highest educational level achieved, was categorized as: (1) low - junior general secondary education or lower (International Standard Classification of Education [ISCED] level 0, 1 or 2); (2) middle - secondary vocational education and senior general secondary education (ISCED level 3 or 4); and (3) high - higher vocational education or university (ISCED level 5 or 6).[12] Smoking status was categorized into never, former, and current smoker. Medication use was binary classified and obtained from the question "Do you use medicine that has been prescribed by a doctor?". Daily energy, lipids, carbohydrate, and alcohol intakes were estimated from a semi-quantitative self-reported food frequency questionnaire (FFQ) by using the 2011 Dutch food composition database (NEVO).[13] The FFQ was developed and validated by Wageningen University to assess the intake of 110 food items over the last month.[14,15]

Laboratory measurements

For analyses of lipids and glucose, blood samples were drawn in the morning between 8:00 and 10:00 am after a period of overnight fasting at baseline. Serum levels of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were measured with an enzymatic colorimetric method, while low-density lipoprotein cholesterol (LDL-C) was measured with an enzymatic method and total triglycerides (TG) was measured with a colorimetric UV method, all on a Roche Modular P chemistry analyzer (Roche, Basel, Switzerland). Fasting blood glucose was measured using a hexokinase method. HbA1c was determined in whole blood (EDTA-anticoagulated) by means of turbid metric inhibition immunoassay on a Cobas Integra 800 CTS analyzer (Roche Diagnostics Netherland BV, Almere, The Netherlands). The presence of T2D at baseline was defined by a self-administrated questionnaire, i.e., "Do you have T2D", together with the diagnostic criteria by the American Diabetes Association (ADA), i.e., a fasting plasma glucose level of 7.0 mmol/L or higher, or a HbA1c level lower than 6.5%.[16]

Analyses of FA were carried out using EDTA-plasma samples collected at baseline and stored at −80 °C until at the Department of Laboratory Medicine of the University Medical Center Groningen, The Netherlands. The methodology was described by Hoving et al..[17] In short, total lipids were extracted by the method of Folch et al., using 6 mL of chloroform-methanol (2:1) and a 200 µL EDTA-plasma sample.[18] Then, a shortened version of the method of Kaluzny et al. was used to isolate plasma cholesterol esters (CE), TG, and PL, using aminopropyl SPE columns for the separation (Isolute, Biotage).[19] FA were transmethylated with methanolic-HCL into fatty acid methyl esters (FAME). The samples were extracted with hexane and eventually redissolved into 100 µL hexane. 100 µL of internal standards for the quantification of FA in CE (17:0) (50.1 mg/100 mL chloroform-methanol, 2:1 v/v), and in TG (19:0) (19.9 mg/100 mL chloroform-methanol, 2:1 v/v), both obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands), were added before isolation of classes. For the quantification of FA in PL, 100 µL of free FA 19:0 (50.0 mg/100 mL methanol), obtained from Larodan (Solna, Sweden), was added after isolation of lipid classes as an internal standard. To prevent fatty acid oxidation, 100 µL Butylated Hydroxytoluene (1 g/100 mL methanol) from Sigma-Aldrich (Zwijndrecht, The Netherlands) was added.

A special assay was developed to be able to separate trans-C18:1n7 from cis-C18:1n7: aliquots of 2 µL were injected into an Agilent model 6890 gas chromatography equipped with a 200 m × 0.25 mm polar column (CP Select for FAME) and detected with an Agilent 7683 series flame ionization detector. FAME was identified by comparing retention times with those of known standards (Supelco 37 component FAME mix (Sigma-Aldrich)). A detailed description of the assay can be found elsewhere.[20] Firstly, FA were measured in a pilot of 96 participants to investigate whether the FA of interest were detectable in plasma CE, TG, and PL. Compared to plasma TG and PL, potentially interesting FA were less detectable in plasma CE. Subsequently, we decided to continue with plasma TG and PL. The precision of the measurements was tested by calculating the variation coefficients from 10 replicate quality-control samples (pooled plasma samples). Circulating FA investigated in this study, i.e., C16:0, cis-C16:1n7, cis-C18:1n7, C18:0, and cis-C18:1n9, as well as the covariate, total n3-PUFA (α-Linolenic acid, Eicosapentaenoic acid, Docosapentaenoic acid, and Docosahexaenoic acid), had variation coefficients $\leq$15%. Circulating FA in plasma PL and TG were expressed as relative percentages of total FA in PL and TG respectively (mol%).

24 h urine samples were also collected at baselines and stored at −80 °C until analysis. Creatinine excretion was calculated by multiplying creatinine concentration in the urine by the urine volume of a single 24 h urine collection. Creatinine in urine was determined using a Roche enzymatic assay, which is traceable to IDMS (Roche, Basel, Switzerland).

Clinical endpoints

The outcome of this study was all-cause mortality. Data on mortality were obtained from the municipal register in December 2020.

Statistical analyses

The distribution of all baseline data was examined visually by viewing histograms and by comparing mean and median values. Continuous data are presented as the mean ± standard deviation (SD) when normally distributed or median and interquartile range (IQR) for non-normally distributed variables. Categorical variables are presented as percentages. Between-group differences were assessed by ANOVA, Kruskal-Wallis, and chi-square two-sided tests for normally distributed continuous data, non-normally distributed continuous data, and categorical data, respectively. Separately, we analyzed baseline characteristics according to tertiles of concentrations of cis-C18:1n7 in plasma PL and TG.

In our primary analysis, Cox proportional hazard models were built to assess the effect of circulating levels of cis-C18:1n7 on all-cause mortality. The proportional hazard assumption was checked by examination of scaled Schoenfeld residuals. The risk of all-cause mortality was presented as per 1-SD increase. Various Cox regression models were built to adjust for possible covariates. The first model was univariate with only the FA; the other models were all multivariable, with model 2 adjusted for age and sex; model 3 adjusted for age, sex, BMI, SBP, education, and smoking status; and model 4 adjusted for age, sex, presence of T2D, and medication use. Additional models were built as sensitivity analyses: model 5 adjusted for age, sex, TG, total cholesterol, and LDL-C; model 6 adjusted for age, sex, and energy intake; model 7 adjusted for age, sex, carbohydrate intake, and alcohol intake; model 8 adjusted for age, sex, and circulating n3-PUFA, as n3-PUFA may also inhibit the production of circulating DNL-related FA by inhibiting the conversion of malonyl-CoA to these FA;[21] model 9 adjusted for age, sex, and circulating palmitic acid (%); model 10 adjusted for age, sex, and BMI; Model 11 adjusted for age, sex, BMI, and 24 h urinary creatinine; and model 12 adjusted for age, sex, and 24 h urinary creatinine.

In the secondary analysis, the same cox proportional hazard models were built for circulating other DNL-related FA, i.e., C16:0, cis-C16:1n7, C18:0, and cis-C18:1n9, to compare the strengths of the associations with cis-C18:1n7. We did not adjust for multiple comparisons, given the prespecified hypothesis for circulating cis-C18:1n7 and all-cause mortality, but exercised caution when interpreting results unrelated to the primary hypotheses. Meanwhile, close attention was paid to internal consistency and findings of others; and appropriate weight was given to the interpretation of biological plausibility based on known pathophysiology, biochemistry, and molecular genetics.

Additionally, we used Cox regression analyses with restricted cubic splines (RCS) with three knots for each FA to test for potential non-linearity of the prospective association of circulating cis-C18:1n7 and other DNL-related FA with all-cause mortality. Non-linearity was not observed for all DNL-related FA; thus, linear hazard ratio plots were drawn for all DNL-related FA using the R-package "rms".

Table 1 Baseline characteristics of participants.

	Total (n = 850)
Male,%	50.2
Age, years	53 ± 15
BMI, kg/m2	26.0 ± 4.1
Presence of T2D,%	5.1
Smoking status,%	
Current smoker	15.3
Former smoker	41.8
Never smoker,	43.0
Education,%	
High	34.6
Middle	32.3
Low	33.1
SBP, mmHg	126.0 ± 17.1
DBP, mmHg	73.4 ± 9.7
Medication use,%	56.0
Energy intake, kcal/d	1971.3 ± 625.1
Lipids intake, g/d	73.0 (57.5–91.3)
Carbohydrate intake, g/d	228.7 ± 76.9
Alcohol intake, g/d	6.2 (1.3–12.6)
Laboratory measurements	
Total cholesterol, mmol/L	5.2 ± 1.0
HDL cholesterol, mmol/L	1.5 ± 0.4
LDL cholesterol, mmol/L	3.3 ± 0.9
Total triglycerides, mmol/L	1.0 (0.8–1.4)
Glucose, mmol/L	5.1 ± 0.7
HbA1c,%	5.6 ± 0.4
Plasma creatinine, μmol/L	75.8 ± 13.6
Urinary creatinine, mmol/24h	13.0 ± 3.9
Circulating fatty acids in plasma PL	
C16:0, mol%	31.2 ± 1.8
C16:1n7, mol%	0.6 ± 0.2
C18:0, mol%	13.3 ± 1.3
C18:1n7, mol%	1.2 ± 0.2
C18:1n9, mol%	8.8 ± 1.4
n3-PUFA, mol%	4.1 (3.4–4.9)
Circulating fatty acids in plasma TG	
C16:0, mol%	26.8 ± 3.6
C16:1n7, mol%	3.2 ± 1.9
C18:0, mol%	4.8 ± 1.6
C18:1n7, mol%	2.0 ± 0.4
C18:1n9, mol%	33.3 ± 5.7
n3-PUFA, mol%	2.6 (2.1–3.3)

*PL: phospholipids, TG: triglycerides, C16:0: palmitic acid, C16:1n7: palmitoleic acid, C18:0: stearic acid, C18:1n7: cis-vaccenic acid, C18:1n9: oleic acid. Missing data: Glucose (0.7%), Education (1.5%), medication (0.7%), energy, alcohol, carbohydrate, and lipids intakes (10.1%).

Potential interactions were also explored by age, sex, BMI, and presence of T2D by including interaction terms with each FA. Missing data on covariates (Glucose: 0.7%, Education: 1.5%, medication: 0.7%, energy intake: 10.1%, alcohol intake: 10.1%, carbohydrate intake: 10.1%, and lipids intakes:10.1%) were imputed through multiple imputation with chained equation in Stata.

Table 2 Association of cis-C18:1n7 (cis-vaccenic acid) in plasma phospholipids (PL) and triglycerides (TG) with all-cause mortality per 1-SD increase.

n_{events}/population=34/850	PL		TG	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Model 1	1.47 (1.08–2.00)	0.01	1.23 (0.89–1.68)	0.2
Model 2	1.60 (1.13–2.25)	0.007	1.32 (0.92–1.89)	0.1
Model 3	1.68 (1.19–2.39)	0.004	1.68 (1.17–2.42)	0.005
Model 4	1.52 (1.07–2.16)	0.02	1.18 (0.81–1.74)	0.4

Multivariable-adjusted Cox proportional-hazards regression analyses between circulating fatty acids in DNL pathway and risk of all-cause mortality(n_{events} = 34); Model 1:univariate, Model 2: model 1+age+sex; Model 3: model 2+BMI+SBP+education+smoking status; Model 4: model 2 + T2D+use of medication;. n_{events}: number of events.

Statistical significance of the Hazard Ratio (HR) for all-cause mortality was defined as 2-tailed α=0.05. All analyses were carried out using Stata (STATA version 13.1; StataCorp, Texas, USA) or RStudio (version 1.1.463; RStudio Inc., Boston, USA).

Results

Baseline characteristics of participants are shown in Table 1. Mean age was 53 ± 15 years, and 49.7% of the participants was female. Educational levels were almost evenly distributed among participants, with 33.1%, 32.3%, and 34.6% having low, middle, and high education, respectively, and 15.3% were never smokers. Mean BMI was 26.0 ± 4.1 kg/m^2. The average carbohydrate and alcohol intakes were 228.7 gs and 6.2 gs per day. Around 5% of the participants had T2D, whereas more than 50% took prescribed medicine. The mean and median levels of HDL-C and TG were 1.5 ± 0.4 mmol/L and 1.0 (0.8–1.4) mmol/L, respectively. Mean levels of cis-C18:1n7 were 1.2 ± 0.2 mol% and 2.0 ± 0.4 mol% in PL and TG, respectively (Table 1). In comparison with plasma TG, plasma PL had higher levels of C16:0 and C18:0 but lower levels of C16:1n7, cis-C18:1n7, and C18:1n9 (Supplementary Figure S2).

During a median follow-up of 9.3 (IQR: 5.4–10.8) years, 34 (4.0%) participants had died. According to our primary analysis, in plasma PL, 1-SD increase in circulating cis-C18:1n7 was significantly associated with an elevated risk of all-cause mortality in the univariate model (HR [95% CI]:1.47 [1.08–2.00], p = 0.01; Table 2, Model 1). The association remained statistically significant after adjustment for age and sex (1.60 [1.13–2.25], p = 0.007; Table 2, Model 2). Further adjustments for plasma lipids profile (TG, TC, and LDL-C), energy intake, carbohydrate and alcohol intake, circulating n3-PUFA and C16:0, or other covariates did not attenuate the magnitude of the association substantially (p<0.02 for all, Table 2 and Supplementary Table S1). In plasma TG, 1-SD increase in circulating cis-C18:1n7 was also positively associated with all-cause mortality, though statistically significant associations were only observed after adjustments for age, sex, BMI, blood pressure, education, and smoking status (1.68 [1.17–2.42], p = 0.005; Table 2, Model 3). Despite the insignificant p-values found between circulating plasma TG cis-C18:1n7 and all-cause mortality in the univariate and several multivariate models, the strengths of the associations with all-cause mortality were stronger in plasma PL than those in plasma TG (Table 2 and Supplementary Table S1).

Of the secondary analysis, in plasma PL and TG, no consistent significant association was found between circulating other DNL-related FA (C:16:0, C16:1n7, C18:0, and C18:1n9) and all-cause mortality. Besides the insignificant associations, the strengths of the associations were also weaker compared with those of cis-C18:1n7 (Supplementary Table S1 and S2).

In further analyses, we found no indication of non-linearity among all the associations (Supplementary Figure S3). There also was no evidence that associations of circulating DNL-related FA with all-cause mortality varied by age, sex, BMI, and presence of T2D (data not shown).

Discussion

Our study has primarily investigated the relationships between circulating cis-C18:1n7 and all-cause mortality in a general population. In this prospective cohort of Dutch adults with normal HDL-C and TG,[22] higher levels of cis-C18:1n7 in plasma PL were positively associated with all-cause mortality. Compared with our secondary analysis of the associations of circulating other DNL-related FA with all-cause mortality, we observed a greater strength of association between circulating cis-C18:1n7 and all-cause mortality.

Cis-C18:1n7 is a monounsaturated and non-essential n7 fatty acid, which can be found in Sea Buckthorn (*Hippophae rhamnoides*) oil,[23] endogenously synthesized through DNL, and can also be derived from the intestinal flora.[9] Trans-C18:1n7, the stereoisomer of cis-C18:1n7, is considered a biomarker for dairy fat intake and has been linked to positive health impacts.[8,20] Thanks to the newly developed assay,[20] we could study the health effect of circulating cis-C18:1n7 apart from trans-C18:1n7, and our results supported the separation of cis- and trans-C18:1n7 because we observed a negative health impact of circulating cis-C18:1n7. Extra caution is needed when comparing our results with previous literature because there is a limited description and evidence to support

that circulating C18:1n7 or cis-C18:1n7 mentioned in those studies was truly separated from trans-C18:1n7.[9,24,25]

In accordance with our results, Lai et al. reported positive associations between circulating plasma PL cis-C18:1n7 with all-cause mortality and non-CVD mortality in a population with elevated CVD risks; more specifically, they reported a positive association of circulating cis-C18:1n7 in plasma PL with cancer mortality within non-CVD mortality.[2] In line, another cross-sectional study observed a positive association between erythrocyte C18:1n7 and breast cancer.[24] Limited and inconsistent epidemiological studies are available regarding circulating plasma cis-C18:1n7 and cardiovascular endpoints. One study found that circulating plasma PL cis-C18:1n7 was associated with a higher risk of sudden cardiac arrest but not of total coronary heart disease (CHD), fatal CHD, or nonfatal myocardial infarction in older adults.[26] On the contrary, Djoussé et al. showed that each SD of circulating plasma PL cis-C18:1n7 was associated with a 41% lower risk of heart failure of male physicians with antecedent CHD,[27] but not of heart failure risk among those who were generally healthy.[28] Moreover, circulating cis-C18:1n7 was inversely associated with CHD risk per SD increase in the same population when measured in red blood cells.[25] Extra caution is needed when interpreting the contradictory results because of the single-sex population or different compartments where FA was measured.[25,28] On the other hand, because of the dominant Caucasian ethnicity, we did not observe any difference in renal function (data not shown) as in the other study that reported a positive association between cis-C18:1n7 and reduced eGFR in Chinese-Americans.[9]

It is difficult to explain the etiology of the positive association between circulating plasma PL cis-C18:1n7 and all-cause mortality observed in this study. However, when regarding the mentioned observational and the fact that circulating cis-C18:1n7 was associated with a beneficial cardiovascular risk profile in our cohort (Supplementary Table S3 and S4), we speculate that the association between circulating cis-C18:1n7 and all-cause mortality observed here might be driven by a higher risk for cancer mortality. Meanwhile, in plasma PL, circulating cis-C18:1n7 was positively correlated with C16:0, C16:1n7, and C18:1n9, and negatively correlated with C18:0 (Supplementary Table S5); a higher level of cis-C18:1n7 could plausibly indicate a more active DNL in general. As cis-C18:1n7 is distal in DNL pathway compared to C16:0, C16:1n7, and C18:0, it might better predict DNL activity, which also explained the stronger magnitude of association observed between circulating cis-C18:1n7 and all-cause mortality compared to other DNL-related FA. In other words, a more active DNL, reflected by a higher level of cis-C18:1n7, might also be related to a higher risk of mortality. Alternatively, the positive association observed between circulating cis-C18:1n7 and all-cause mortality could reflect detrimental effects of increased concentrations of palmitate, with a compensatory increase of circulating cis-C18:1n7 as a consequence of increasing stearoyl-CoA desaturase (SCD) and ELOVL activity,[29] to allow for conversion of palmitate into nontoxic MUFA, such as cis-C18:1n7.

Despite the restricted evidence from observational studies, some mechanistic studies might provide insights into our findings regarding circulating cis-C18:1n7 and all-cause mortality. In cancer cell studies, cis-MUFA, including cis-C18:1n7, reversed the anti-proliferative effect of a SCD inhibitor, suggesting that SCD controlled the overall rate of proliferation in lung cancer cells through MUFA-mediated activation of lipid synthesis.[30] Similarly, a dietary intervention study conducted in mice found that impaired tumor SCD activity resulted in lower levels of MUFA, including cis-C18:1n7, can slow tumor growth.[31] In addition, cis-C18:1n7 is a direct product of ELOVL5 elongation, and lipid elongation through ELOVL5 was identified as a pro-tumorigenic metabolic pathway in prostate cancer.[32] The findings of these mechanistic studies are in agreement with an earlier observational study in which it was found that cis-C18:1n7 was associated with cancer mortality.[2]

Besides cis-C18:1n7, several epidemiological studies support potential harms and risks of circulating or tissue levels of C16:0, C16:1n7, and C18:1n9, although we did not observe any statistically significant associations in this study. A pooled analysis of prospective cohort studies has shown positive relations between these three FA and incident T2D.[3] Furthermore, circulating C16:0, C16:1n7, and C18:1n9 have been positively associated with CVD events and all-cause mortality[2,5,6] in older adults with and without elevated CVD risks. The underlying biological mechanisms could be related to inflammation,[33] apoptosis,[34] induction of steatosis,[34,35] and beta-cell dysfunction[36] as observed in experimental studies. More specifically, a study suggested that the roles of circulating C16:0, C16:1n7, and C18:1n9 are more common to diseases of aging in general, rather than highly disease-specific mechanisms, because of similar risks for both CVD and non-CVD mortality, and more specifically, positive associations with dementia mortality and trauma/fracture mortality.[2] As the current study population was middle-aged, these findings might explain the insignificant results found for circulating C16:0, C16:1n7, C18:0, and C18:1n9, with all-cause mortality in the current study.

Circulating DNL-related FA could be influenced by various determinants. Studies have suggested that plasma PL reflects chronic FA intake and could better predict clinical outcomes than plasma TG,[37-40] whereas plasma TG reflects acute FA intake.[41] Thus, FA from plasma PL might be a good reflection of endogenous synthesis because it eliminates FA from TG and cholesteryl esters that are more likely to be sourced from recent dietary ingestion, but this is still unclear.[41] Interestingly, we only observed a statistically significant association between circulating plasma PL cis-C18:1n7 with all-cause mortality, but not in plasma TG. Since plasma PL is mostly synthesized endogenously and TG is synthesized both endogenously and exogenously from the diet, it seems that DNL, rather than the direct dietary intake of these FA, plays a more critical role in contributing to a higher

risk of mortality, especially considering that cis-C18:1n7 is mostly produced endogenously from C16:1n7.

The current study has several strengths. First, the general population-based cohort used in our study improved the generalizability of our finding to younger adults, as suggested by previous studies.[2,6] Second, we have extended studies that evaluated the relation between DNL pathway activity and mortality by investigating the association between circulating cis-C18:1n7 and mortality, which has rarely been done in previous studies because of the difficulties in separating trans-C18:1n7 from cis-C18:1n7. Third, we included two different plasma lipids fractions, PL and TG, separately to compare different FA pools, which supported the previous hypothesis that plasma PL, compared to plasma TG, is better suited to investigate the associations between circulating DNL-related FA and clinical outcomes. Limitations should also be considered. First, the small sample size and low mortality rate have induced power issues, making it difficult to establish statistically significant associations. Therefore, additional studies in larger sample sizes are required to validate these relationships. Second, the possibility of residual confounding by imprecisely measured or unknown factors cannot be excluded. For example, we could not include physical activity as a covariate because the proportion of missing data (50.5%) was beyond the ideal imputation range. However, similar results were found when we further adjusted for 24 h urinary creatinine excretion as a biomarker for muscle mass to represent physical activity in the sensitivity analysis. Additionally, we were unable to identify all fatty acids that might interact with the enzymes involved in DNL, which made it not possible to adjust for potential confounding by an impact of these fatty acids on rate of DNL. Third, it cannot be excluded that circulating FA investigated in this study could derive from diet in addition to DNL, as we did not perform isotope labeling studies to trace and quantify the rate of DNL and the activity of its related elongases and desaturases. Finally, our study population consisted predominantly of Caucasian participants living in the Netherlands, a country known for its relatively high intake of dairy products, which calls for prudence to extrapolate our results to different populations concerning ethnicity and dietary patterns.

Conclusion

A higher level of plasma PL cis-C18:1n7 was positively associated with all-cause mortality in a general population. Furthermore, circulating cis-C18:1n7 seems to be a better marker to reflect DNL activity than other DNL-related FA because of the stronger magnitude of association with all-cause mortality. These findings encourage further investigations that could replicate and verify our results, as well as experimental studies to explore potential mechanisms explaining the observed associations between circulating DNL-related FA, especially cis-C18:1n7, and clinical outcomes.

Declaration of Competing Interest

The authors declared no conflict of interest.

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Author Contributions

Y.Z., S.J.L.B., G.J.N., and I.J.R. designed research; I.G.P. conducted research; F.A.V., M.R.H.F., and I.M. provided essential reagents and materials; Y.Z. and I.J.R. analyzed data and performed statistical analysis; Y.Z., G.J.N., and S.J.L.B. wrote the paper; I.G.P., F.A.V., M.R.H.F., and I.M. critically reviewed the manuscript; Y.Z. had primary responsibility for final content.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jacl.2022.07.003.

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