

# Plasma Phospholipid Concentration of *Cis*-Palmitoleic Acid and Risk of Heart Failure

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**Background**—Although plasma palmitoleic acid has been positively associated with blood pressure, inflammation, and insulin resistance, its association with heart failure has not been investigated. We assessed whether plasma phospholipid *cis*-palmitoleic acid was associated with heart failure risk.

**Methods and Results**—This ancillary study of the Physicians' Health Study used a risk set sampling method to select 788 matched pairs. For each case of incident heart failure, we randomly selected a control among subjects that were free of heart failure and alive at the time of index case diagnosis and matched on age, year of birth, race, and time of blood collection. Plasma phospholipid fatty acids were measured using gas chromatography. Heart failure was ascertained using annual follow-up questionnaire and validated in a subsample. In a multivariable conditional logistic regression, odds ratios (95% CI) for heart failure were 1.0 (ref), 1.06 (0.75–1.48), 1.20 (0.85–1.68), and 1.58 (1.11–2.25) across consecutive quartiles of *cis*-palmitoleic acid (*P* for trend 0.009). Each SD increase in plasma *cis*-palmitoleic acid was associated with 17% higher odds of heart failure (95% CI: 2% to 33%) in a multivariable model. In a secondary analysis, each SD increase of log-stearoyl-coA desaturase activity (16:1n-7/16:0 ratio) was positively associated with the risk of heart failure (odds ratio: 1.14 [95% CI: 1.00 to 1.29]), whereas oleic acid and *cis*-vaccenic acid concentrations were not related to heart failure risk.

**Conclusions**—Our data showed a positive association between plasma phospholipid *cis*-palmitoleic acid and heart failure risk in male physicians. (*Circ Heart Fail.* 2012;5:703-709.)

**Key Words:** heart failure ■ epidemiology ■ fatty acids ■ *cis*-palmitoleic acids ■ risk factors

Owing in part to a better survival after myocardial infarction and rising prevalence of obesity, diabetes mellitus, and hypertension, heart failure (HF) burden and costs are still on the rise in the United States.<sup>1–5</sup> Despite state-of-the-art management of symptomatic HF, its mortality remains extremely high with <50% of patients surviving beyond 5 years after the onset. Hence, it remains critical to identify novel risk factors and biomarkers that could lead to novel treatment approaches, primary prevention, and risk stratification. The human body can use excess carbohydrate to synthesize fatty acids via de novo lipogenesis (DNL) pathway.<sup>6</sup> Fatty acids in the DNL or lipid synthesis including palmitic acid (16:0), *cis*-palmitoleic acid (16:1n-7), oleic acid (18:1n-9), *cis*-vaccenic acid (18:1n-7), and so on, have been reported to influence risk factors for HF, such as adiposity,<sup>7–9</sup> hypertension,<sup>10</sup> diabetes mellitus,<sup>11,12</sup> inflammation,<sup>7,13</sup> and cardiovascular mortality.<sup>14,15</sup> Other reports found no relation between plasma palmitoleic acids and coronary heart disease<sup>16,17</sup> or

diabetes mellitus.<sup>7</sup> In animal experiments, a mixture of fatty acids, including palmitoleic acid, stearic acid, and palmitic acid, was shown to induce cardiac growth and increased left ventricular mass,<sup>18</sup> suggesting that specific fatty acids from the DNL might influence the development of HF. However, limited data are available in humans on the effects of plasma phospholipids *cis*-palmitoleic acid and other fatty acids in the DNL on the risk of HF. Data from the Atherosclerosis Risk in Community (ARIC) study<sup>19</sup> reported no association between total phospholipid palmitoleic acid (*cis* and *trans* combined) and incident HF. Because *trans* palmitoleic acid is derived from naturally occurring dairy/ruminant *trans* fatty acids<sup>20</sup> and its consumption has not been associated with a higher risk of cardiovascular diseases,<sup>21</sup> it is possible that the results from the ARIC study were influenced by a neutral effects of *trans* palmitoleic acid on HF. The current ancillary study sought to examine the association between phospholipid *cis*-palmitoleic acid and HF risk among United States male physicians. Because

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stearoyl coA desaturase drives the desaturation of palmitic acid (16:0) to palmitoleic acid (16:1n7), we hypothesized that stearoyl coA desaturase activity would be positively associated with HF risk. Thus, in a secondary analysis, we assessed the relation of stearoyl coA desaturase activity as well as other fatty acids in the DNL with HF risk.

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### Methods

#### Study Population

Participants in this ancillary study analyses are members of the Physicians Health Study (PHS) I who provided baseline (1982) blood samples. The PHS I is a completed randomized, double-blind, placebo-controlled trial designed to study low-dose aspirin and  $\beta$ -carotene for the primary prevention of cardiovascular disease and cancer.<sup>22,23</sup> Current analyses used a prospective nested case-control design to select subjects. For each case of HF, we randomly selected a control among participants who were alive and free of HF at the time of diagnosis of the index case. Each control was matched on age at randomization (within 1 year), race (white versus non-white), year of birth (within 1 year), and time of blood collection (within 288 days). A total of 788 incident HF with matching controls were used for the current study. Each participant signed an informed consent and the institutional review board at Brigham and Women's Hospital approved the study protocol.

#### Measurement of Plasma Phospholipid Fatty Acids

The fatty acid profile was measured in plasma using the method previously described by Cao et al.<sup>24</sup> For the extraction of plasma phospholipid fatty acids, 0.3 mL of plasma is mixed with 0.7 volume of 0.9% saline. Lipids are extracted from plasma with a mixture of chloroform:methanol (2:1, v/v), and cholesterol, triglycerides, and phospholipid subclasses are separated on a silica thin-layer chromatography plate in a solvent mixture of petroleum ether, diethyl ether, and glacial acetic acid (80:20:1, v/v/v). The band of phospholipids is harvested for the formation of methyl esters. Fatty acid methyl esters are prepared with 1.5 mL of 14% boron trifluoride in methanol, incubated at 80°C for 90 minutes, and extracted with petroleum ether. The final product is dissolved in heptane and injected onto a capillary Varian CP7420 (Agilent Technologies, Santa Clara, CA) 100-m column with a Hewlett Packard 5890 gas chromatograph equipped with a HP6890A autosampler (Hewlett Packard Corporation, Palo Alto, CA). The gas chromatograph is configured for a single capillary column with a flame ionization detector and interfaced with HP chemstation software. Adequate separation of fatty acid methyl esters is obtained during a 80-minute period with an initial temperature of 190°C for 25 minutes. The temperature is increased to 240°C at a rate of 2°C/min and held for 5 minutes. Fatty acid methyl esters from 14:0 through 24:1n-9 are separated, identified, and expressed as percent of total fatty acids. The following coefficients of variations were obtained on 30 blind duplicates: linoleic acid=1.7%;  $\alpha$  linolenic acid 3.0%; arachidonic acid=3.2%; eicosapentaenoic acid=5.1%; docosapentaenoic acid=3.8%; docosahexaenoic acid=4.9%; 16:0=1.1%; 16:1n7cis=1.8%; 18:0=3.5%; and 18:1n7=2.9%.

#### Ascertainment of Incident HF in the PHS

We used annual follow-up questionnaires to ascertain cardiovascular end points including HF in the PHS. A detailed description of the validation of self-reported HF in the PHS has been previously published.<sup>25</sup> Briefly, 2 independent physicians reviewed medical records on a subsample of participants who reported a diagnosis of HF on a follow-up questionnaire. The positive predictive value of self-reported HF using medical records review was 91% and agreement between the 2 reviewers was excellent ( $\kappa=92\%$ ).

#### Other Variables

All relevant covariates were assessed at baseline (1982) to be consistent with the time of blood collection and exposure assessment. Demographic information was obtained through self-reports. At baseline, each subject provided information on exercise, smoking (never, former, and current smoker), and alcohol intake (rarely/never, 1–3 per month, 1 per week, 2–4/week, 5–6/week, daily, and 2+/day). Self-reported baseline weight and height were used to compute body mass index (weight in kilograms divided by height in meter squared). Information on comorbidity including diabetes mellitus, atrial fibrillation, hypercholesterolemia, valvular heart disease, coronary heart disease, and hypertension was collected at baseline and through follow-up questionnaires. In the PHS I, a food frequency questionnaire was administered between 1999 and 2000. Nutrients were derived using food composition databases from Harvard University supplemented by manufacturers' information. Validity of such food frequency questionnaire has been reported elsewhere.<sup>26</sup>

#### Statistical Analysis

Using the distribution of plasma phospholipid *cis*-palmitoleic acid in the control series (n=788), we created quartiles of *cis*-palmitoleic acid. Means and percentages of baseline characteristics of the study participants are presented according to quartiles of *cis*-palmitoleic acid. We used conditional logistic regression to estimate the RR of HF using the lowest quartile of *cis*-palmitoleic acid as reference category. The initial model adjusted for matching variables (race, age, time of blood collection, and year of birth), a second model also controlled for body mass index, prevalent hypertension, coronary artery disease, atrial fibrillation, diabetes mellitus, and plasma phospholipid 18:0 and marine omega-3 fatty acids (20:5n-3, 22:5n-3, and 22:6n-3). A final model controlled for vigorous physical activity (>1 per week versus <1 per week), smoking (never, former, and current smokers), and alcohol consumption (rarely/never, <1, 1–6, and 7+ drinks/week). To obtain a *P* value for linear trend, we used quartile of *cis*-palmitoleic acid as ordinal variable in the regression model. We repeated above analyses using *cis*-palmitoleic acid as a continuous variable and estimated RR of HF associated with 1 SD (0.1467% of total plasma phospholipid fatty acids in the control series) increase of *cis*-palmitoleic acid. In secondary analyses, we evaluated the association between stearoyl-coA activity, defined as a ratio of product-to-substrate (16:1n-7/16:0 and 18:1n-9/18:0),<sup>8,14</sup> and the risk of HF. In addition, we explored relations of *cis*-vaccenic acid (18:1n-7) and *cis*-oleic acid (18:1n-9) with HF risk using analytical approach outlined for *cis*-palmitoleic acid. Lastly, we used total protein and carbohydrate estimates from a food questionnaire to explore their influence on the observed association and repeated main analyses for HF with and without prior coronary artery disease (myocardial infarction, coronary bypass, or angioplasty). All analyses were performed using SAS (SAS Institute, Inc., version 9.3, Cary, NC) and the  $\alpha$  level was set at 0.05. All *P* values were 2-sided.

### Results

The mean age (SD) was 58.7 (8.0) years (range, 40 to 82) among 1576 study participants. In the control series, mean plasma concentration of *cis*-palmitoleic acid was 0.32% of total plasma phospholipids (range, 0.04% to 2.22%). Compared with individuals in the lowest quartile, those with higher concentration of *cis*-palmitoleic acid had a higher prevalence of hypertension and atrial fibrillation, consumed less alcohol, smoked more cigarettes, had higher concentration of marine omega-3 fatty acids and stearoyl-coA desaturase activity, and were more likely to exercise vigorously (Table 1). *Cis*-palmitoleic acid was weakly associated with total, trans, omega-3, and other major fatty acids (Table I in the online-only Data Supplement). Compared with controls, cases had higher body mass index, were less likely to exercise

**Table 1. Characteristics of 1576 Male Physicians According to Quartiles of Plasma Phospholipid Palmitoleic Acid**

Characteristics	Quartiles of Plasma Phospholipid Palmitoleic Acid (% of Total Fatty Acids)			
	Q1 (Low) [0.039–0.227]	Q2 [0.228–0.280]	Q3 [0.281–0.359]	Q4 (high) [0.360–2.217]
	N=364	N=376	N=394	N=442
Age, y	58.4±8.1	58.2±8.0	59.1±8.1	59.1±7.9
Body mass index, kg/m <sup>2</sup>	24.8±2.7	25.1±2.8	25.2±2.9	25.6±3.2
Plasma cis 16:1 n-7*	0.19±0.03	0.25±0.02	0.32±0.02	0.50±0.16
Plasma DHA*	3.15±1.04	3.03±0.89	3.13±1.00	2.91±0.94
Plasma DPA*	0.80±0.20	0.85±0.30	0.84±0.20	0.87±0.24
Plasma EPA*	0.70±0.38	0.70±0.34	0.78±0.58	0.84±0.37
Linoleic acid*	22.3±2.8	22.0±2.8	21.6±2.6	20.2±2.5
Plasma 16:0*	24.9±1.5	25.1±1.3	25.4±1.4	26.4±1.4
Plasma 18:0*	14.3±1.2	14.0±1.1	13.9±1.2	13.5±1.3
Steaoyl-coA desaturase activity				
Ratio 16:1n-7/16:0	0.008±0.001	0.010±0.001	0.013±0.001	0.019±0.006
Ratio 18:1n-9/18:0	0.52±0.10	0.55±0.08	0.56±0.08	0.64±0.11
Prevalent diabetes, %	6.0	4.5	5.3	6.3
Prevalent CHD, %	16.2	13.6	17.0	14.3
Atrial fibrillation, %	3.6	2.9	4.3	4.8
Hypertension, %	27.3	25.7	35.5	35.2
Current smoking, %	10.4	12.2	8.4	13.6
Never smokers, %	52.5	43.4	48.6	37.6
Current drinkers, %	80.1	84.8	79.1	90.9
Vigorous exercise, %	70.2	75.1	75.4	73.6

DHA indicates docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; CHD, coronary heart disease. Data are presented as means±SD or percentages. Few participants had missing data on hypertension (n=10), smoking (n=2), alcohol use (n=6), and physical activity (n=10).

\*Plasma phospholipid fatty acids expressed as percentage of total plasma phospholipids

vigorously, were more likely to be current smoker, and had a higher prevalence of diabetes mellitus, hypertension, atrial fibrillation, and coronary heart disease (Table 2). In a conditional logistic regression model adjusting for matching factors, odds ratios (95% CI) for HF were 1.0 (ref), 1.05 (0.78 to 1.41), 1.23 (0.91 to 1.66), and 1.51 (1.12 to 2.03) across consecutive quartiles of plasma phospholipid *cis*-palmitoleic acid (*P* trend 0.003, Table 3). Additional adjustment for body mass index, prevalent hypertension, atrial fibrillation, diabetes mellitus, coronary heart disease, plasma phospholipid 18:0, 20:5n-3, 22:5n-3, 22:6n-3, smoking, alcohol intake, and exercise had minimal influence on the results (*P* for trend 0.009, Table 2). Each SD increase of *cis*-palmitoleic acid was associated with a 17% higher risk of HF (95% CI: 2% to 33%) (Table 2). Additional adjustment for plasma phospholipid total saturated and trans fatty acids had minimal affect on the association (odds ratio: 1.45 [95% CI: 1.02 to 2.79] comparing the highest with the lowest quartile and *P* for trend 0.03). The positive association of *cis*-palmitoleic acid with HF was restricted to HF without antecedent coronary disease (myocardial infarction or coronary bypass/angioplasty) with multivariable adjusted odds ratios of 1.0 (ref), 1.07 (0.71 to 1.62), 1.20 (0.80 to 1.82), and 1.56 (1.02 to 2.39), *P* for trend 0.03. Corresponding values for HF with antecedent coronary disease were 1.0 (ref), 0.91 (0.44 to 1.91), 0.84 (0.44 to 2.03), and 1.32 (0.62 to 2.79), *P* trend 0.45.

In secondary analyses, we examine the relation of steaoyl-coA desaturase activity and other fatty acids in the DNL (*cis*-oleic acid [18:1n-9] and *cis*-vaccenic acid [18:1n7]) with the risk of HF and found a positive association with 16:1n-7/16:0 ratio, but not with any of the other biomarkers examined (Table 4). Each SD of log-transformed ratio of 16:1n-7/16:0 was associated with an odds ratio of 1.14 (95% CI: 1.00–1.29, Table 3).

## Discussion

In the current study, we demonstrated a positive association between plasma phospholipid *cis*-palmitoleic acid and steaoyl-coA desaturase activity and the risk of HF in US male physicians, after adjustment for major risk factors for HF and determinants of DNL including alcohol intake. In contrast, phospholipid concentrations of *cis*-vaccenic acid and oleic acid were not associated with HF risk.

To the best of our knowledge, this is the first large study to demonstrate a positive association between plasma phospholipid *cis*-palmitoleic acid and the risk of HF in a large cohort. In a cross-sectional study, HF patients were shown to have higher plasma levels of 16:1n-7 compared with controls (1.88% versus 1.40% weight of total fatty acids, *P*<0.001).<sup>27</sup> In the ARIC study,<sup>19</sup> the highest quintile of plasma phospholipid palmitoleic acids was associated with 67% higher risk of incident HF compared with the lowest quintile after controlling for age and sex (RR=1.67 [95% CI: 1.10 to

**Table 2 Characteristics of Cases and Controls**

Characteristics	Cases (N=788)	Controls (n=788)
Age at randomization, y	58.9±8.0	58.8±8.0
Age at blood collection, y	58.7±8.0	58.7±8.1
Year of birth	1923.6±8.0	1923.6±8.0
Year of blood collection	1982.2±0.4	1982.1±0.3
Body mass index, kg/m <sup>2</sup>	25.8±3.2	24.6±2.5
Plasma cis 16:1 n-7*	0.33±0.15	0.32±0.15
Plasma DHA*	3.03±0.95	3.07±0.99
Plasma DPA*	0.83±0.24	0.85±0.24
Plasma EPA*	0.75±0.46	0.76±0.41
Linoleic acid*	21.3±2.8	21.6±2.8
Plasma 16:0*	25.6±1.6	25.4±1.5
Plasma 18:0*	14.0±1.2	13.8±1.2
Stearoyl-coA desaturase activity		
Ratio 16:1n-7/16:0	0.013±0.005	0.012±0.005
Ratio 18:1n-9/18:0	0.57±0.11	0.57±0.11
Race (% white)	95.8	95.8
Prevalent diabetes, %	7.9	3.3
Prevalent CHD, %	21.7	8.8
Atrial fibrillation, %	5.5	2.4
Hypertension, %	37.6	24.8
Current smoking, %	13.6	8.9
Never smokers, %	41.0	49.4
Current drinkers, %	82.9	85.1
Vigorous exercise, %	71.4	75.8

DHA indicates docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; CHD, coronary heart disease. Data are presented as means±SD or percentages. Few participants had missing data on hypertension (n=10), race (n=52), smoking (n=2), alcohol use (n=6), and physical activity (n=10).

\*Plasma phospholipid fatty acids expressed as percentage of total plasma phospholipids.

2.52]). However, adjustment for body mass index, systolic blood pressure, antihypertensive medication, lipids, diabetes mellitus, smoking, alcohol consumption, energy intakes, education, and physical activity attenuated the association (ie, RR=1.10 [95% CI: 0.70–1.73] when comparing extreme

quintiles). The difference between our findings and ARIC data merits some comments. First, we examined cis-isoform of plasma phospholipid palmitoleic acid, whereas ARIC<sup>19</sup> data reported findings of cis and trans 16:1n-7. Trans-(16:1n-7) is a biomarker of dairy intake, which may exert favorable effect on HF risk factors including diabetes mellitus.<sup>28</sup> The average age in our study was about 5 years higher than in the ARIC (54 years for men and 53 years for women in ARIC versus 59 years in PHS), but ARIC had fewer HF events (n=197) than PHS (n=788). It is possible that the ARIC study had limited statistical power to detect small effect of 16:1n-7. Lastly, the fact that our study consisted of highly educated male healthcare professionals with a higher prevalence of healthful lifestyle factors (low prevalence of smoking, comorbidity, and obesity, high prevalence of physical activity, and so on) than the general population in ARIC could partially account for the difference in the results.

In our secondary analysis, we observed a positive association between plasma *cis*-palmitoleic acid and HF without antecedent coronary heart disease and no relation between *cis*-palmitoleic acid and HF with antecedent coronary events. This suggests that *cis*-palmitoleic acid might not influence the risk of HF via adverse effects on coronary heart disease. However, such a hypothesis is not consistent with our previous work showing a higher risk of coronary heart disease with higher concentrations of red blood cell *cis*-palmitoleic acid.<sup>15</sup> It is also possible that the discrepancy between HF and coronary disease outcomes when related to *cis*-palmitoleic acid might be partially explained by our inability to correctly classify HF with and without antecedent coronary events.

Our findings of a positive association between stearoyl-coA desaturase activity (16:1n-7/16:0 ratio) and HF is contrary to the ARIC report,<sup>19</sup> which did not show an association (*P* for trend across quintile 0.68 and RR=1.06 [95% CI: 0.66 to 1.69] comparing the fifth to the first quintile of 16:1n-7/16:0 ratio). We examined for the first time the relation of *cis*-vaccenic acid (18:1n-7) with HF risk and found no association. This is contrary to our hypothesis of an inverse relation between 18:1n-7 and HF risk owing to the conversion of plasma 16:1n-7 to 18:1n-7 via elongase activity in the DNL pathway. A lack of such an association could be because of the relatively small proportions of both *cis*-vaccenic and

**Table 3 Odds Ratios for Heart Failure by Quartiles or Per SD Increase of Plasma Phospholipid Palmitoleic Fatty Acids in the Physicians' Health Study**

Cis 16:1n-7 Quartiles [Range]	Cases	Odds Ratio (95% CI) for Heart Failure		
		Model 1*	Model 2†	Model 3‡
Q1 (low) [0.039–0.227]	169	1.0	1.0	1.0
Q2 [0.228–0.280]	177	1.05 (0.78–1.41)	1.10 (0.79–1.53)	1.06 (0.75–1.48)
Q3 [0.281–0.359]	197	1.23 (0.91–1.66)	1.20 (0.86–1.68)	1.20 (0.85–1.68)
Q4 (high) [0.360–2.217]	245	1.51 (1.12–2.03)	1.52 (1.08–2.14)	1.58 (1.11–2.25)
<i>P</i> for trend		0.003	0.014	0.009
Per SD (0.1467%) increase of plasma cis 16:1n-7		1.13 (1.02–1.26)	1.13 (1.00–1.28)	1.17 (1.02–1.33)

\*Model 1 adjusted for matching variables.

†Model 2 adjusted for matching variables plus body mass index, prevalent hypertension, coronary disease, atrial fibrillation, and diabetes mellitus, plasma phospholipid 18:0 and marine omega-3 fatty acids (EPA, DHA, and DPA).

‡Model 3 adjusted for variables in model 2 plus smoking (never, former, current smokers), vigorous physical activity (at least weekly vs less frequent), and alcohol consumption (rarely/never, <1, 1–6, and 7+ drinks per week).

**Table 4 Odds Ratios for Heart Failure Per SD Increase of Stearoyl-CoA Desaturase Activity, Plasma Phospholipid Oleic Acid, and Cis-Vaccenic Acid in the Physicians' Health Study**

One SD Increase of	Odds Ratio (95% CI) for Heart Failure		
	Model 1*	Model 2†	Model 3‡
<i>Cis</i> -vaccenic acid (18:1n-7) [SD=0.208]	0.88 (0.79–0.97)	1.02 (0.91–1.15)	1.03 (0.91–1.17)
<i>Cis</i> -oleic acid (18:1 n-9) [SD=1.06]	1.09 (0.98–1.21)	1.10 (0.97–1.25)	1.11 (0.97–1.26)
Stearoyl-coA desaturase activity			
Estimated by 16:1n-7-to-16:0 ratio [SD=0.358]*	1.13 (1.01–1.25)	1.11 (0.99–1.26)	1.14 (1.00–1.29)
Estimated by 18:1n-9-to-18:0 ratio [SD=0.178]*	1.02 (0.92–1.13)	1.04 (0.91–1.19)	1.04 (0.90–1.20)

\*Model 1 adjusted for matching variables; log-transformed ratio of product-to-substrate to estimate stearoyl-coA desaturase activity.

†Model 2 adjusted for matching variables plus body mass index, prevalent hypertension, coronary disease, atrial fibrillation, and diabetes mellitus, plasma phospholipid 18:0 and marine omega-3 fatty acids (EPA, DHA, and DPA).

‡Model 3 adjusted for variables in model 2 plus smoking (never, former, current smokers), vigorous physical activity (at least weekly vs less frequent), and alcohol consumption (rarely/never, <1, 1–6, and 7+ drinks per week). Stearoyl-coA desaturase activity (16:1n-7/16:0) was also adjusted for 18:0 and 18:1n-9/18:0 was also adjusted for 16:0.

*cis*-palmitoleic acids to total phospholipids with a median (interquartile range) of 0.28% (0.23–0.36) of total plasma phospholipid fatty acids for 18:1n-7 and 1.42% (1.30–1.54) for 16:1n-7 among controls.

### Biologic Mechanisms

What possible biologic mechanisms could help establish a causal relation between *cis*-palmitoleic acids and HF risk? Animal experiments have shown that python heart grows in mass by 40% after ingestion of a large meal<sup>29,30</sup>; such physiologic changes are accompanied by an increase in plasma free fatty acids.<sup>18</sup> Infusion of fasted pythons with a mixture of myristic, palmitic, and palmitoleic acids was as effective as feeding itself in cardiac hypertrophy.<sup>18</sup> Furthermore, treatment of mice with a similar mixture of fatty acids led to increased left ventricular mass as well as increased cardiomyocyte cross-sectional area.<sup>18</sup> Although these animal results may not be transferable to humans, they suggest that fatty acids in the DNL (ie, palmitoleic acid) could modulate left ventricular geometry and contribute to the development of HF. Palmitoleic acid may increase the risk of HF via hypertension. Zheng et al<sup>10</sup> reported an increased prevalent hypertension per interquartile increment of cholesterol ester 16:1n-7 (OR=1.31 [95% CI: 1.18 to 1.44]) as well as increased incident hypertension before adjustment for baseline blood pressure (OR=1.26 [95% CI: 1.11 to 1.42]). However, our data did not support a major role of baseline hypertension on the association of *cis*-palmitoleic acid with HF, as there was no meaningful difference in regression models with and without adjustment of hypertension (data not shown). Lastly, heightened inflammation could be a potential mechanism by which 16:1n-7 increases the risk of HF as Petersson et al<sup>13</sup> reported a positive correlation between serum cholesteryl ester levels of 16:1n-7 and C-reactive protein in men. In mice, palmitoleic acid downregulated mRNA expression of proinflammatory genes (tumor necrosis factor  $\alpha$  and resistin) in white adipose tissue and lipogenic genes (ie, fatty acid synthase or stearoyl-coA desaturase-1) in the liver.<sup>31</sup>

It is not clear whether palmitoleic acid may influence the risk of HF via its effects on coronary heart disease or diabetes mellitus. In a nested case-control study, total plasma phospholipid 16:1n-7 was not associated with coronary

heart disease in men (OR: 1.08 [95% CI: 0.77 to 1.57] per SD of 16:1n-7).<sup>17</sup> Administration of palmitoleic acid in mice was associated with enhanced whole-body glucose disposal and improved insulin sensitivity.<sup>31,32</sup> In humans, some<sup>28,33</sup> but not other<sup>7</sup> investigators have reported insulin-sensitizing effects of palmitoleic acid. Our group has reported a positive association between red blood cell *cis*-palmitoleic acid and the risk of coronary heart disease.<sup>15</sup> Additional studies are needed to elucidate biologic mechanisms lending support to a causal relation between *cis*-palmitoleic acid and HF risk. The absence of an association between *cis*-vaccenic acid and HF suggests that if our findings are confirmed in future studies, pharmacological interventions targeting elongase activity (to metabolize *cis*-palmitoleic acid [16:1n-7] to *cis*-vaccenic acid [18:1n-7]) might be of interest to mitigate such a risk. Our working hypothesis is that a positive association between stearoyl-coA desaturase activity and HF is because of conversion of a neutral palmitic acid (16:0) to a detrimental *cis*-palmitoleic acid (16:1n7).

### Strengths and Limitations

The large number of participants, 22+ years of follow up, and the standardized methods of comorbidity ascertainment and measurement of plasma phospholipids fatty acids are major strengths of this study. In addition, we were able to minimize confounding by age, race, time of blood collection, and birth cohort effect through matching between cases and control series. Lastly, our findings remained robust upon additional adjustment for alcohol (and perhaps estimates of carbohydrate and protein intake), factors that influence DNL. However, the fact that all participants were male physicians, most of whom were whites, limits generalization of current findings to the general population, other ethnic groups, and women. Furthermore, we did not have data to allow us to further classify HF based on cause or left ventricular systolic function (systolic or diastolic HF). HF diagnosis was primarily self-reported by participants who were physicians and despite a 91% positive predictive value of self-reported HF against validation via medical record review in a subsample, we cannot completely exclude HF misclassification (over- or under-reporting). Such misclassification, if present, is more likely to be nondifferential with respect to plasma fatty acids

assays and would likely have led to an underestimation of the true effect. The lack of nutrients at baseline precluded a full adjustment for other dietary determinants of *cis*-palmitoleic acid including carbohydrate and protein consumption. In a secondary analysis, we controlled for total proteins and carbohydrates collected about 17 years after *cis*-palmitoleic assessment and observed similar findings. Of note is that we cannot assume that dietary patterns remained constant during 17 years of follow up; further more, some participants were deceased or had missing data on nutrients and these limitations could have led to inadequate adjustment. Lastly, this ancillary study could not support the cost of plasma fatty acid measurement on all PHS subjects. Nonetheless, the use of risk-set technique to select control subjects randomly makes our findings generalizable to the entire PHS I population.

In summary, our data showed a positive association between plasma phospholipid *cis*-palmitoleic acid and stearyl-coA desaturase (16:1n-7/16:0 ratio) and the risk of HF in US male physicians. Avoidance of diet that favors DNL (ie, diet with excess carbohydrate, protein, and alcohol) could be recommended to minimize high plasma *cis*-palmitoleic acids if our findings were confirmed in other article.

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### Disclosures

None.

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### CLINICAL PERSPECTIVE

De novo lipogenesis (endogenous production of fatty acids including palmitoleic acid) may influence blood pressure, inflammation, insulin resistance, and coronary disease. However, it is unknown whether fatty acids generated by the de novo lipogenesis influence the risk of heart failure. Furthermore, the association of key enzymes involved in the de novo lipogenesis with heart failure risk has not been investigated. Using a nested case-control design, we studied 788 incident heart failure cases and 788 matched controls from the Physicians' Health Study. Each case of heart failure was matched to its control on age, year of birth, race, and time of blood collection. Plasma phospholipid fatty acids were measured using gas chromatography. Heart failure was ascertained using annual follow-up questionnaire and validated in a subsample. In a multivariable conditional logistic regression, odds ratios (95% CI) for heart failure were 1.0 (ref), 1.06 (0.75 to 1.48), 1.20 (0.85 to 1.68), and 1.58 (1.11 to 2.25) across consecutive quartiles of *cis*-palmitoleic acid (*P* for trend 0.009). This association was restricted to heart failure without antecedent coronary heart disease. In a secondary analysis, each SD increase of log-stearoyl-coA desaturase activity was associated with a 14% higher risk of heart failure (95% CI: 1.00 to 1.29). In contrast, oleic acid and *cis*-vaccenic acids were not associated with heart failure risk. If confirmed in other studies, our study might provide a novel pharmacological target to lower the risk of heart failure caused by a heightened endogenous fatty acid production.

### Plasma Phospholipid Concentration of *Cis*-Palmitoleic Acid and Risk of Heart Failure

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## **SUPPLEMENTAL MATERIAL**

**Online Table 1.** Spearman Correlation coefficients between plasma phospholipid cis-palmitoleic acid and other fatty acids

<b>Fatty acids</b>	<b>Correlation coefficient</b>	<b>P value</b>
Eicosapentaenoic acid (20:5 n-3)	0.17	<0.0001
Docosahexaenoci acid (22:6 n-3)	-0.098	0.006
Docosapentaenoic aci (22:5 n-3)	0.048	0.17
Palmitic acid (16:0)	0.40	<0.0001
Stearic acid (18:0)	-0.25	<0.0001
Cis-vaccenic acid (18:1n-7)	0.15	<0.0001
Cis-oleic acid (18:1 n-9)	0.49	<0.0001
Plasma total trans fatty acids	-0.29	<0.0001
Plasma total saturated fatty acids	0.17	<0.0001