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Plasma fatty acids, oxylipins, and risk of myocardial infarction: the Singapore Chinese Health Study[S]

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Abstract We aimed to examine the prospective association between plasma FAs, oxylipins, and risk of acute myocardial infarction (AMI) in a Singapore Chinese population. A nested case-control study with 744 incident AMI cases and 744 matched controls aged 47–83 years was conducted within the Singapore Chinese Health Study. Nineteen plasma FAs and 12 oxylipins were quantified using MS. These were grouped into 12 FA clusters and 5 oxylipin clusters using hierarchical clustering, and their associations with AMI risk were assessed. Long-chain n-3 FAs [odds ratio (OR) = 0.67 per SD increase, 95% confidence interval (CI): 0.53–0.84, $P < 0.001$] and stearic acid (OR = 0.65, 95% CI: 0.44–0.97, $P = 0.03$) were inversely associated with AMI risk, whereas arachidonic acid (AA) was positively associated with AMI risk (OR = 1.25, 95% CI: 1.03–1.52, $P = 0.02$) in the multivariable model with adjustment for other FAs. Further adjustment for oxylipins did not substantially change these associations. An inverse association was observed between AA-derived oxylipin, thromboxane (TX)B_2, and AMI risk (OR = 0.81, 95% CI: 0.71–0.93, $P = 0.003$). Circulating long-chain n-3 FAs and stearic acid were associated with a lower and AA was associated with a higher AMI risk in this Chinese population. The association between the oxylipin TXB_2 and AMI requires further research.—Sun, Y., Hiromi W. L. Koh, H. Choi, W-P. Koh, J-M. Yuan, J. W. Newman, J. Su, J. Fang, C. N. Ong, and R. M. van Dam. **Plasma fatty acids, oxylipins, and risk of myocardial infarction: the Singapore Chinese Health Study. *J. Lipid Res.* 2016.** 57: **1300–1307.**

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Dietary FAs have been implicated in the etiology of coronary heart disease (CHD) (1). Results from cohort studies suggest that long-chain n-3 PUFAs are associated with a lower CHD risk and *trans*-fats are associated with a higher CHD risk (2, 3). Higher consumption of n-6 PUFAs has also been associated with a lower cardiovascular risk (4, 5), but concerns remain about their pro-inflammatory and pro-thrombotic potential through synthesis of oxidized metabolites (6, 7).

Oxylipins are a group of oxidized metabolites of PUFAs metabolized through various enzymatic pathways. These include the more conventional subclass of 20-carbon oxylipins derived from arachidonic acid [AA, 20:4(n-6)], named eicosanoids, and other more recently identified oxylipins that are metabolized via lipoxygenase and cytochrome P450 pathways. These oxylipins have hormone-like effects on inflammation, vasoconstriction, and blood clotting in experimental studies (8), and may thus act as mediators of the effects of dietary FAs on cardiovascular

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Abbreviations: AA, arachidonic acid; AMI, acute myocardial infarction; CHD, coronary heart disease; CI, confidence interval; CRP, C-reactive protein; DHET, dihydroxyeicosatrienoic acid; DiHOME, dihydroxyoctadeca(mono)enoic acid; HbA1c, glycosylated hemoglobin; HEPE, hydroxyeicosapentaenoic acid; KETE, ketoeicosatetraenoic acid; LA, linoleic acid; LTE_4, leukotriene E_4; OR, odds ratio; QC, quality control; SCHS, Singapore Chinese Health Study; SFA, saturated FA; TX, thromboxane.

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health. Changes in dietary FA precursors could change the balance of oxylipins with different biological effects (9, 10), which may affect the risk of CHD. However, epidemiological data on oxylipins and CHD risk are sparse. In a study of human carotid atherosclerotic plaques, the concentration of HETEs was significantly higher in symptomatic plaques, as compared with asymptomatic plaques (11). In addition, higher plasma HETE concentrations were associated with CHD in two case-control studies (12, 13). However, data from larger prospective studies with a more comprehensive assessment of oxylipins are currently not available. We therefore examined the association between plasma concentrations of FAs and oxylipins and risk of acute myocardial infarction (AMI) in a case-control study nested within the Singapore Chinese Health Study (SCHS) cohort. To circumvent the problem of high collinearity among these metabolites, we have devised an automated approach to group the highly correlated metabolites into clusters and study their associations with AMI risk.

MATERIALS AND METHODS

SCHS

SCHS is a population-based prospective cohort study of 63,257 Chinese men and women (Hokkien or Cantonese dialect group) aged 45–74 years residing in public housing estates, where 86% Singaporeans resided at the time of recruitment. Recruitment and assessment of baseline diet, lifestyle, and medical history took place from 1993 to 1998. To date, follow-up surveys have been conducted twice (1999–2004; 2006–2010) to update information on use of tobacco and alcohol, medical history, and menopausal status. Biospecimens were collected from 28,439 subjects during 1994–2005. The cohort has been followed up for morbidity and mortality through record linkage with Singapore Registry of Births and Deaths, Hospital Discharge Database, and Singapore Cancer Registry. Data on education, BMI, physical activity, smoking, alcohol intake, and history of diabetes and hypertension were assessed using interviewer-administered standardized questionnaires (14, 15). This study was approved by the Institutional Review Boards of National University of Singapore and University of Pittsburgh. All participants gave informed consent.

Nested case-control study

We have established a nested case-control study including 744 incident AMI cases and 744 matched controls. These cases and controls were selected from the SCHS participants who provided blood and did not have a history of physician-diagnosed CHD or stroke reported at blood draw or ascertained through linkage with the Hospital Discharge Database before blood draw (International Classification of Diseases-9 codes: 410–414, 427, 428, 430–434, 438). The eligible cases for the present study were incident nonfatal or fatal AMI that occurred during follow-up from blood collection through December 31, 2010 via linkage with three databases (see supplementary text). Using a risk-set sampling approach (16), we randomly chose one control for each case. Control subjects were alive and free of CHD at the time of the AMI diagnosis or death of the index case. The matching criteria included gender, dialect group (Hokkien, Cantonese), date of birth (±5 years), date of recruitment (±2.5 years), and date of blood collection (±6 months).

Measurement of CVD risk factors

During home visits in 1994–2005, systolic and diastolic blood pressure was measured and blood samples were collected in heparin tubes. The blood specimens were separated into various blood components, including plasma, serum, red blood cells, and buffy coat, and were stored in freezers at −80°C for a mean of 11.5 ± 1.8 years before the lab analyses (17). Conventional biochemical risk factors of CHD, including total, HDL, and LDL (directly measured) cholesterol, triglycerides, C-reactive protein (CRP), creatinine, and glycosylated hemoglobin (HbA1c), were measured. Detailed measurement methods and coefficients of variation of these cardiovascular risk factors are described in the supplementary text.

Measurement of plasma FAs and oxylipins

Nineteen plasma FAs, covering major saturated FAs (SFAs), monounsaturated FAs, and PUFAs, were measured in a targeted mode using GC-MS/MS on an Agilent 7890 GC system (Shanghai, China) equipped with a G7000B QQQ triple quadrupole mass detector and an auto sample injector. FAs from both free and esterified (triglycerides, phospholipids, cholesterol esters) fractions were measured in total.

Nineteen plasma-free oxylipins with potential roles in inflammation, blood pressure regulation, and platelet degranulation (supplementary Table 1) were targeted to be measured using reversed-phase LC-MS with an Agilent 1290 ultra-pressure liquid chromatograph (Waldbronn, Germany) coupled to electrospray ionization with iFunnel Technology on a triple quadrupole mass spectrometer. Twelve out of the 19 targeted oxylipins could be reliably quantified in the majority of the samples, and were thus included in the current analysis. These included: seven AA-derived oxylipins [thromboxane (TX)B_2, leukotriene E_4 (LTE_4), 5-HETE, 12-HETE, 15-HETE, 5-ketoeicosatetraenoic acid (5-KETE), 14,15-dihydroxyeicosatrienoic acid (14,15-DHET)]; four linoleic acid [LA, 18:2(n-6)]-derived oxylipins [9-HODE, 13-HODE, 9,10-dihydroxyoctadeca(mono)enoic acid (9,10-DiHOME), 12,13-DiHOME]; and one EPA [20:5(n-3)]-derived oxylipin [5-hydroxyeicosapentaenoic acid (5-HEPE)]. The free fraction of plasma AA, a major precursor of oxylipins, was also assessed in the same assay.

Samples were analyzed in 76 batches for FAs and 51 batches for oxylipins. Cases and their matched controls were included in the same batch. All procedures were conducted with lab personnel being blinded to the case control status of the samples. Pooled human plasma was used for quality control (QC) samples that were included at regular intervals (one QC in every 10 samples). There were three and four QCs in each batch for FA and oxylipin analysis, respectively. The experimental details and the coefficients of variation of the measured FAs and oxylipins are listed in the supplementary text and supplementary Table 2.

Statistical analysis

Our nested case-control study initially included a total of 744 matched case-control pairs. Due to lack of plasma samples, we could not obtain reliable oxylipin values in 17 matched pairs of cases and controls. After exclusion of these participants, 727 matched case-control pairs remained for the current analysis.

Prior to any statistical analysis, we first performed normalization of the quantitative data in three steps. First, we adjusted for batch-effect by dividing the molar concentration of each individual FA and oxylipin by the average concentration of their corresponding compound in the QC samples for each batch. Second, the concentrations for FAs and oxylipins were converted to proportions of their respective total concentrations. For oxylipins, this approach of using proportions of total concentrations is less

established, so sensitivity analysis was conducted on the raw concentrations as well. In the last step, the proportions (or the raw concentrations in the sensitivity analysis) were log-transformed, centered by mean, and scaled to have a standard Gaussian distribution for each compound.

We then devised a procedure to automatically group highly correlated compounds together to avoid multi-collinearity problems. We performed hierarchical clustering of the Pearson correlation matrix (agglomerative clustering with Euclidean distance) for the FAs and the oxylipins, respectively, using the data of the controls only. We determined the predictor groups using the Dynamic Tree Cut procedure (18) implemented in R (19), which adaptively selected a sparse set of clusters given a dendrogram (clustering tree) obtained by initial clustering results. Once the compounds were grouped into clusters, each cluster was subjected to further inspection, where it was divided into sub-clusters if the average pairwise correlation coefficient between the compounds was less than 0.3 within that cluster. In our data, the Dynamic Tree Cut procedure reported 12 initial clusters, and the second-stage inspection resulted in 17 clusters: 12 for FAs and 5 for oxylipins. Given the final cluster configuration, a centroid concentration pattern for each cluster was computed by the average of all its member compounds, i.e.:

$$C_i = \frac{1}{n_i}\sum_{j=1}^{n_i} x_j \text{ for } i = 1,\ldots,c \text{ and } j = 1,\ldots,n_i$$

where x_j denotes the batch-adjusted normalized log-concentration of compound j belonging to cluster i, c denotes the total number of clusters, and n_i denotes the total number of compounds present in cluster i.

Demographic characteristics, established cardiovascular risk factors, and plasma concentrations of FAs and oxylipins in the cases and controls were compared using univariate conditional logistic regression.

The association between these 17 generated clusters and the risk of AMI was assessed using conditional logistic regression models by computing the multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs). In the multivariable model, in addition to control for the five matching factors as a result of the matching design and use of conditional logistic regression analysis, we further adjusted for age (years), hours of fasting before blood collection (<2 h; 2 h to <8 h; ≥8 h), education level (none; primary school; secondary school or above), cigarette smoking (never; ex-smoker; current smoker with <13 cigarettes/day; current smoker with ≥13 cigarettes per day), alcohol consumption (grams per day), physical activity (0 h per week; <4 h per week of moderate and <2 h per week of strenuous activity; ≥4 h per week of moderate or ≥2 h per week of strenuous activity), BMI (kilograms per square meter), history of hypertension (yes/no), and history of diabetes (yes/no). The regression models were compared with or without adjustment for other FA clusters, oxylipin clusters, and conventional cardiovascular risk factors.

For specific FAs that showed significant attenuation after adjustment for the conventional cardiovascular risk factors, cross-sectional association between the FAs and the cardiovascular risk factors was assessed by multivariable linear regression among the control subjects.

The initial data transformation, normalization, and cluster generation analyses were carried out using R version 3.0.3. The rest of the statistical analyses were conducted using Stata version 11 (StataCorp, College Station, TX). All P values reported are two-sided. $P < 0.05$ is considered to be statistically significant.

RESULTS

Demographic characteristics

Characteristics of the 727 incident AMI cases and 727 matched controls included in the current analysis are shown in **Table 1**. The mean age at blood collection was 66 years (range 47–83 years), and 65% of participants were male. Participants who developed AMI were more likely to be current smokers, had no regular physical activity, a higher BMI, and often a history of hypertension and diabetes. Incident AMI cases had higher blood pressure, LDL cholesterol, triglycerides, creatinine, CRP, and HbA1c, but lower HDL cholesterol at baseline. Plasma concentrations of stearic acid (18:0), EPA [20:5(n-3)], DHA [22:6(n-3)], and TXB_2 were significantly lower in the cases than in the controls, whereas plasma concentrations of oleic acid [*cis*-18:1(n-9)] and 5-KETE were significantly higher in the cases. Molar concentrations of plasma total FAs and free oxylipins in cases and controls are presented in supplementary Table 3.

Correlation structure

As shown in the correlation heat map (**Fig. 1**), both FAs and oxylipins showed a high degree of correlation within their respective classes. Figure 1 illustrates the configuration of five oxylipin clusters (clusters 1–5) and 12 FA clusters (clusters 6–17), based on the automated clustering procedure. Some clusters were aggregated as expected based on their common food sources: cluster 7 consisted of three odd-chain FAs and eicosadienoic acid (20:2), all found in dairy products; cluster 11 included the two long-chain n-3 PUFAs, which are mainly found in fish and shellfish. Other clusters may reflect endogenous conversions: cluster 9 included two mid-chain n-6 PUFAs, cluster 13 included two mid-chain n-3 PUFAs, and cluster 17 included two 20-carbon FAs. The correlation among the oxylipins also reflected endogenous synthesis pathways: five of the AA-derived oxylipins were grouped into cluster 2 (two HETEs and LTE_4) and cluster 5 (TXB_2 and 12-HETE); and four LA-derived oxylipins were grouped into cluster 3 (two HODEs) and cluster 4 (two DiHOMEs). Oxylipins derived from the same FA were positively correlated, whereas oxylipins derived from AA and LA tended to be inversely correlated. Most of the correlations between FAs and oxylipins were weak and not statistically significant. The strongest correlation was observed between EPA and its oxidized product, 5-HEPE ($r = 0.30$). In contrast, plasma free AA, unlike plasma total AA, was significantly correlated with four of its oxidized metabolites (5-HETE, 15-HETE, 14,15-DHET, and 5-KETE) (supplementary Fig. 1). Overall, the multi-collinearity was considerably reduced after the compounds were clustered, as shown in the correlation heat map, indicating the orthogonality of clusters (supplementary Fig. 2).

Multivariable-adjusted association with AMI risk

Table 2 shows the association between plasma FA and oxylipin clusters and risk of AMI. In the multivariable model (model 2), long-chain n-3 PUFAs and stearic acid

TABLE 1. Distribution of selected characteristics, cardiovascular risk factors, and plasma concentrations of FAs and oxylipins in AMI cases and their matched controls, the SCHS

	Cases	Controls	P_{diff} [a]
N	727	727	
Age (years)	66.1 (7.9)	66.0 (7.8)	NA (matched)
Gender (% male)	64.79	64.79	NA (matched)
Education (% secondary and above)	25.86	28.20	0.27
Current smoker (%)	31.09	22.28	<0.001
No regular physical activity[b] (%)	74.42	67.40	0.004
BMI (kg/m^2)	23.2 (3.1)	22.9 (2.9)	0.05
Energy intake (kcal/day)	1,607 (589)	1,610 (586)	0.93
Ethanol intake (% never drinkers)	79.23	75.65	0.10
History of hypertension (%)	46.77	36.73	<0.001
History of diabetes (%)	24.76	12.10	<0.001
Cardiovascular risk factors			
Systolic blood pressure (mmHg)	149.1 (23.7)	141.4 (21.7)	<0.001
Diastolic blood pressure (mmHg)	83.0 (11.8)	81.1 (11.0)	0.002
LDL cholesterol (mmol/l)	3.34 (0.87)	3.19 (0.81)	0.001
HDL cholesterol (mmol/l)	1.29 (0.30)	1.35 (0.33)	<0.001
Triglycerides (mmol/l)	1.68 (0.70)	1.57 (0.68)	0.001
Creatinine (umol/l)	70 (58–84)	68 (57–80)	0.003
CRP (mg/l)	1.5 (0.7–3.5)	1.1 (0.5–2.4)	<0.001
HbA1c (%)	6.0 (5.7–7.1)	5.8 (5.5–6.2)	<0.001
Plasma total FAs (% total)			
15:0	0.07 (0.06–0.09)	0.07 (0.06–0.09)	0.08
16:0	20.05 (18.82–21.4)	19.88 (18.58–21.15)	0.06
16:1(n-7)	1.76 (1.27–2.34)	1.74 (1.32–2.26)	0.86
17:0	0.14 (0.12–0.16)	0.14 (0.12–0.16)	0.08
17:1(n-8)	0.08 (0.06–0.09)	0.08 (0.06–0.09)	0.99
18:0	7.32 (6.26–8.28)	7.5 (6.39–8.45)	<0.001
18:1(n-9) *trans*	0.05 (0.03–0.06)	0.05 (0.04–0.06)	0.13
18:1(n-9) *cis*	21.81 (20.14–23.77)	21.32 (19.46–23.27)	<0.001
18:2(n-6)	36.15 (4.63)	36.41 (4.66)	0.26
18:3(n-6)	0.19 (0.12–0.30)	0.19 (0.13–0.29)	0.61
18:3(n-3)	0.28 (0.21–0.38)	0.29 (0.22–0.38)	0.28
20:0	0.05 (0.04–0.06)	0.05 (0.04–0.06)	0.42
20:1(n-9)	0.11 (0.09–0.12)	0.1 (0.09–0.12)	0.13
20:2(n-6)	0.18 (0.16–0.21)	0.18 (0.16–0.2)	0.11
20:3(n-3)	0.01 (0.01–0.02)	0.02 (0.01–0.02)	0.17
20:3(n-6)	0.88 (0.67–1.14)	0.85 (0.68–1.08)	0.41
20:4(n-6)	7.44 (6.44–8.74)	7.5 (6.47–8.73)	0.79
20:5(n-3)	0.39 (0.31–0.49)	0.41 (0.32–0.52)	<0.001
22:6(n-3)	1.90 (1.37–2.77)	1.98 (1.42–2.98)	0.002
Plasma free oxylipins (% total)			
TXB_2	3.05 (1.33–5.06)	3.09 (1.51–5.56)	0.02
LTE_4	0.78 (0.36–1.74)	0.79 (0.35–1.83)	0.59
5-HETE	2.89 (1.66–4.81)	2.75 (1.66–4.47)	0.44
12-HETE	24.11 (14.03–37.56)	23.26 (13.4–35)	0.47
15-HETE	1.23 (0.71–1.84)	1.16 (0.67–1.75)	0.29
5-KETE	0.35 (0.12–1.15)	0.29 (0.11–0.95)	0.04
14,15-DHET	1.72 (1.06–2.57)	1.48 (0.94–2.42)	0.06
9-HODE	11.11 (7.90–15.25)	11.83 (8.11–16.75)	0.15
13-HODE	14.07 (11.10–17.49)	14.52 (10.88–18.41)	0.64
9,10-DiHOME	13.27 (8.81–19.94)	13.3 (8.68–20.09)	0.54
12,13-DiHOME	14.25 (9.79–21.41)	14.96 (9.89–21.33)	0.96
5-HEPE	0.87 (0.43–1.56)	0.89 (0.41–1.61)	0.70
Free AA (ug/ml)	0.80 (0.59–1.10)	0.79 (0.6–1.09)	0.91

Values are mean (SD) for normally distributed continuous variables, median (interquartile range) for continuous variables not normally distributed, and percentage for categorical variables. Number of missing values: systolic and diastolic blood pressure, 85; triglycerides, 60; HbA1c, 2. NA, not applicable.

[a] P_{diff} was derived from univariate conditional logistic regression. Variables were log transformed if they had skewed distribution.

[b] Only physical activities of moderate or vigorous intensity were compared.

were associated with lower risk of AMI, whereas oleic acid was associated with higher risk of AMI. In model 3, we further adjusted for all other clusters of the same category (FAs or oxylipins) except one major cluster with neutral effects, cluster 6 for FAs and cluster 1 for oxylipins, to avoid multi-collinearity. After adjustment for other FA clusters, similar inverse associations were observed for long-chain n-3 PUFAs (OR = 0.67, 95% CI: 0.53–0.84, $P < 0.001$) and stearic acid (OR = 0.65, 95% CI: 0.44–0.97, $P = 0.03$), whereas the association for oleic acid was no longer significant. AA, which was not substantially associated with AMI risk in model 2, became significantly associated with higher risk of AMI after adjustment for other FA clusters (OR = 1.25, 95% CI: 1.03–1.52, $P = 0.02$). The addition of long-chain n-3 PUFAs was the main contributor to the attenuation of the association

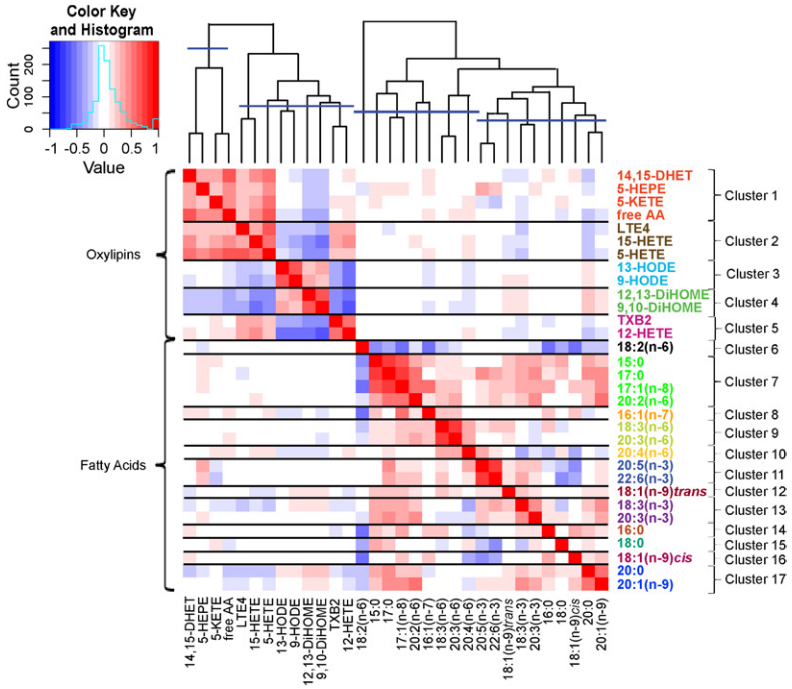

Fig. 1. Correlation heat map among plasma FAs and oxylipins, and cluster generation from the control group.

for oleic acid, and the strengthening of the association for AA. Among the oxylipins, only the cluster of TXB_2 and 12-HETE was significantly associated with lower risk of AMI in the model, with adjustment for other oxylipin clusters (model 3) (OR = 0.80, 95% CI: 0.66–0.98, $P = 0.03$).

Mediation by oxylipins and cardiovascular risk factors

The clusters which were associated with AMI risk in Table 2 were examined further by adjusting for potential biological mediators of their associations (**Table 3**). Adjustment for oxylipin clusters did not substantially attenuate associations for the FA clusters. In contrast, the associations for AA and stearic acid were substantially attenuated after adjustment for cardiovascular risk factors. To explore which specific cardiovascular risk factors might explain this attenuation, we assessed the cross-sectional multivariable-adjusted association between these two FAs and cardiovascular risk factors in the control group. Stearic acid was marginally associated with lower HbA1c, but this association was not statistically significant

TABLE 2. Associations of FA and oxylipin clusters with risk of AMI, the SCHS

			Model 1		Model 2[a]		Model 3[b]	
Cluster	Compounds Included in the Cluster	Description	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
1	14,15-DHET, 5-HEPE, 5-KETE, AA	AA, EPA oxylipins	1.10 (0.95, 1.28)	0.20	1.03 (0.87, 1.22)	0.73	—	
2	LTE_4, 15-HETE, 5-HETE	AA oxylipins	1.06 (0.93, 1.21)	0.41	1.02 (0.87, 1.18)	0.83	1.06 (0.88, 1.26)	0.55
3	9-HODE, 13-HODE	LA oxylipins	0.93 (0.82, 1.04)	0.21	0.96 (0.84, 1.10)	0.59	0.90 (0.77, 1.06)	0.21
4	9,10-DiHOME, 12,13-DiHOME	LA oxylipins	0.98 (0.87, 1.10)	0.70	0.98 (0.86, 1.13)	0.80	0.90 (0.75, 1.09)	0.28
5	TXB_2, 12-HETE	AA oxylipins	0.94 (0.83, 1.06)	0.30	0.92 (0.81, 1.05)	0.23	0.80 (0.66, 0.98)	0.03
6	18:2(n-6)	LA	0.94 (0.83, 1.06)	0.29	0.97 (0.85, 1.11)	0.71	—	
7	15:0, 17:0, 17:1(n-8), 20:2(n-6)	Odd-chain FAs	0.93 (0.77, 1.12)	0.44	0.94 (0.77, 1.15)	0.57	1.05 (0.79, 1.38)	0.75
8	16:1(n-7)	Palmitoleic acid	1.00 (0.90, 1.11)	0.97	1.04 (0.92, 1.17)	0.56	1.02 (0.87, 1.21)	0.78
9	18:3(n-6), 20:3(n-6)	GLA, DGLA	1.04 (0.92, 1.18)	0.54	1.08 (0.95, 1.24)	0.24	1.03 (0.85, 1.24)	0.77
10	20:4(n-6)	AA	0.99 (0.88, 1.11)	0.86	1.02 (0.90, 1.15)	0.75	1.25 (1.03, 1.52)	0.02
11	20:5(n-3), 22:6(n-3)	EPA, DHA	0.70 (0.59, 0.83)	<0.001	0.71 (0.59, 0.85)	<0.001	0.67 (0.53, 0.84)	<0.001
12	18:1(n-9) *trans*	*trans* Elaidic acid	0.90 (0.80, 1.02)	0.09	0.88 (0.78, 1.01)	0.06	0.88 (0.77, 1.01)	0.06
13	18:3(n-3), 20:3(n-3)	ALA, ETE	0.88 (0.76, 1.03)	0.10	0.88 (0.75, 1.04)	0.14	0.96 (0.78, 1.18)	0.71
14	16:0	Palmitic acid	1.13 (0.99, 1.28)	0.06	1.03 (0.89, 1.18)	0.70	1.00 (0.84, 1.19)	0.98
15	18:0	Stearic acid	0.56 (0.41, 0.78)	0.001	0.66 (0.47, 0.92)	0.02	0.65 (0.44, 0.97)	0.03
16	18:1(n-9) *cis*	Oleic acid	1.20 (1.06, 1.35)	0.003	1.14 (1.01, 1.28)	0.03	1.08 (0.93, 1.26)	0.29
17	20:0, 20:1(n-9)	20-Carbon FAs	1.05 (0.89, 1.23)	0.56	1.02 (0.85, 1.22)	0.82	1.06 (0.86, 1.31)	0.57

ORs (95% CIs) were derived from conditional logistic regression models and expressed as per SD increase of each cluster. The FAs are expressed as percentage of total FAs and the oxylipins as percentage of total oxylipins. ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; ETE, eicosatrienoic acid; GLA, γ-linolenic acid.

[a]The conditional logistic regression models included the following additional variables: hours of fasting before blood collection, level of education, cigarette smoking, alcohol consumption, physical activity, BMI, and history of hypertension and diabetes.

[b]In addition to the variables described in footnote *a*, the conditional logistic regression models further included all other oxylipin clusters in models for clusters 2–5 or all other FA clusters in models for clusters 7–17.

TABLE 3. Change in associations of FA and oxylipin clusters with risk of AMI when adding potential biological mediators, the SCHS

	Model with Multivariable Factors[a]		Model with Oxylipin Clusters[b]		Model with CVD Risk Factors[c]	
Exposure Variable	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Clusters						
10 [20:4(n-6)]; AA	1.25 (1.03, 1.52)	0.02	1.22 (1.00, 1.49)	0.05	1.16 (0.93, 1.44)	0.19
11 [20:5(n-3), 22:6(n-3)]; EPA, DHA	0.67 (0.53, 0.84)	<0.001	0.66 (0.53, 0.83)	<0.001	0.67 (0.52, 0.86)	0.002
15 [18:0]; stearic acid	0.65 (0.44, 0.97)	0.03	0.66 (0.44, 0.98)	0.04	0.79 (0.49, 1.28)	0.33
5 [TXB_2, 12-HETE]; AA oxylipins	0.80 (0.66, 0.98)	0.03	—	—	0.84 (0.67, 1.05)	0.12
Individual compounds						
20:5(n-3); EPA	0.74 (0.62, 0.88)	0.001	0.73 (0.61, 0.88)	0.001	0.75 (0.61, 0.91)	0.004
22:6(n-3); DHA	0.75 (0.60, 0.93)	0.008	0.74 (0.59, 0.92)	0.007	0.71 (0.55, 0.91)	0.008
TXB_2; AA oxylipin	0.81 (0.71, 0.93)	0.003	—	—	0.85 (0.73, 1.00)	0.05
12-HETE; AA oxylipin	1.00 (0.82, 1.21)	0.97	—	—	0.99 (0.79, 1.24)	0.91

ORs (95% CIs) were derived from conditional logistic regression models and expressed as per SD increase of exposure variables.

[a]Conditional logistic regression models were adjusted for the covariates listed in model 3 of Table 2: hours of fasting before blood collection, education, cigarette smoking, alcohol consumption, physical activity, BMI, history of hypertension, history of diabetes, other FA clusters (in models for clusters 10, 11, 15, EPA, and DHA) or other oxylipin clusters (in models for cluster 5, TXB_2, and 12-HETE).

[b]In addition to the variables described in footnote *a*, the conditional logistic regression models further included the five oxylipin clusters (clusters 1–5).

[c]In addition to the variables described in footnote *a*, the conditional logistic regression models further included the following additional cardiovascular risk factors: systolic blood pressure, LDL and HDL cholesterol, triglycerides, creatinine, CRP, and HbA1c.

(supplementary Table 4). AA was significantly associated with higher LDL cholesterol, HDL cholesterol, creatinine, and HbA1c, but lower triglycerides.

Sensitivity analyses

We conducted a sensitivity analysis examining individual components of the clusters associated with AMI risk. The two long-chain n-3 PUFAs in cluster 11 were both associated with lower AMI risk, whereas the association between cluster 5 (TXB_2 and 12-HETE) and risk of AMI was solely due to TXB_2 (OR = 0.81, 95% CI: 0.71–0.93, P = 0.003) (Table 3). Sensitivity analyses using the absolute concentration of TXB_2 instead of a proportion and with or without adjustment for other oxylipins showed similar significant associations (OR ranged from 0.80 to 0.87, all $P <$ 0.05). TXB_2 was not significantly associated with cardiovascular risk factors (supplementary Table 3) and its association with AMI risk was not substantially attenuated by adjustment for these risk factors (Table 3).

DISCUSSION

In this prospective nested case-control study of Singapore Chinese, we observed an inverse association between long-chain n-3 PUFAs and stearic acid with risk of AMI, and a positive association for AA after adjustment for other FAs. We also observed an inverse association between TXB_2, an AA-derived oxylipin, and risk of AMI. The association between plasma FAs and AMI was partly mediated by established cardiovascular risk factors, but not by the oxylipins measured.

The association between long-chain n-3 PUFAs and CHD has been extensively examined in previous cohort studies, most of which also showed an inverse association (20). In contrast, plasma stearic acid had a positive or null association with CHD risk in previous studies in Western populations (21). In a Japanese study, the only other study conducted in Asia, plasma stearic acid was inversely associated with CHD risk (22), which was consistent with our findings. The disparity in results for Western versus Asian populations may reflect differences in dietary sources of stearic acid. Stearic acid is highly correlated with other SFAs in Western diet because they share the same predominant food sources, such as red meat and dairy (23). It is therefore difficult to distinguish the effect of stearic acid from other SFAs in a Western diet. In contrast, the major food sources of SFAs in Singapore are plant-based cooking oils and coconut oil/milk (24). The correlation between plasma stearic acid and other even-chain SFAs in our data (r range 0.14–0.20) was weaker than that observed in Western populations, which facilitated examination of the specific association for stearic acid. A lack of detrimental effects of stearic acid is supported by feeding trials which showed that dietary stearic acid did not result in elevated serum cholesterol concentrations or impaired insulin sensitivity, as compared with carbohydrates or monounsaturated FAs (25, 26).

Adipose tissue AA was associated with higher risk of AMI in case-control studies in Costa Rica and Israel (27, 28), which is consistent with our observation for plasma AA. In most other previous studies, no association or an inverse association between AA biomarkers and CHD was observed (21, 29), and few studies adjusted for other FAs. One interesting observation in our analysis is that AA became significantly associated with a higher AMI risk only after adjustment for other FA clusters. Confounding by other FAs can arise if they share similar food sources or if their food sources are

part of the same dietary pattern. It is therefore important to consider such confounding by other FAs in studies of circulating FAs and health outcomes.

There have been concerns about LA intake, as it may be converted to AA endogenously, and AA may increase levels of detrimental oxylipins which increase blood pressure and inflammation (6, 30). However, our data suggest that plasma LA is not associated with AMI risk, and LA is not necessarily associated with higher AA concentrations ($r = -0.06$). In line with this finding, wide variation of dietary LA intake did not substantially affect tissue AA concentrations in a meta-analysis of feeding trials (31). Therefore, it is unlikely that dietary LA increases risk of AMI through conversion to AA. Furthermore, we could not identify potential detrimental effects of AA through increased production of oxylipins. The cardiovascular risk factors that AA was associated with were also not the risk factors plausibly affected by oxylipins. Besides the commonly known pro-inflammatory and pro-aggregatory metabolites, such as the leukotrienes and TXs, AA is also the precursor to other anti-inflammatory or vasodilating metabolites, such as the lipoxins (32) and epoxyeicosatrienoic acids (33). It is therefore difficult to predict the net impact of the entire AA metabolome, likely depending on the differential enzymatic activity for the synthesis of metabolites, as well as the interactions among the metabolites (34). Hence, detailed mechanistic studies are warranted to elucidate potential underlying mechanisms for the direct association between AA and AMI risk.

The pro-thrombotic oxylipin, TXA_2, functions as a transient autocrine, and its biological inactive metabolite, TXB_2, has been used to indirectly measure TXA_2 production (35). The inverse association between TXB_2 is thus unexpected and requires further study. We did not observe substantial association between other oxylipin clusters and AMI risk besides TXB_2. The autocrine and short-lived nature of these oxylipins might account for the lack of association in our long-term prospective study (36). However, we cannot exclude the possibility that other oxylipins or oxylipins within the esterified pool of lipoprotein particles, rather than the free oxylipins that we examined, predict CHD. In addition, the concentrations of TXB_2 and possibly other oxylipins may reflect sample collection, processing, and storage conditions rather than only biological differences in vivo, and the association between oxylipins and AMI in our study should therefore be interpreted with caution.

To our knowledge, our study is the first prospective study of a wider range of oxylipins in relation to AMI risk. In addition, it is among the largest studies of FA biomarkers and AMI in Asia. The values we obtained for FAs and oxylipins were within the range of those reported in earlier studies, suggesting a reliable quantification. Furthermore, the clustering among various FAs and oxylipin families was as expected based on biological knowledge. However, our study also has several potential limitations. First, there may be misclassifications in the measurement of plasma FAs and oxylipins. For example, we only included measurements of FAs and oxylipins at a single point in time, which may not fully capture long-term effects on CHD development. Plasma FAs reflect dietary FA intake of the past 1–2 weeks (37). However, misclassification of FA and oxylipin status was likely to be nondifferential and probably led to weaker associations with AMI risk. Second, we measured only major oxylipins in plasma rather than the full spectrum. Other oxylipins that we did not examine in this study might play a more significant role in mediating certain FAs. Third, the stability of the analytes may deteriorate with long storage time, which may affect the detected levels in the studied samples. The controls were thus individually matched to the cases for time of blood collection to minimize the influence of storage duration. Fourth, as an observational study, our results may have been affected by residual confounding by imperfectly or unmeasured metabolic, genetic, or behavioral risk factors. Finally, our results may have been affected by multiple testing, as we examined 17 clusters as exposure variables. Using Bonferroni correction ($\alpha = 0.05/17 = 0.003$), the association for long-chain n-3 PUFAs remains statistically significant, whereas associations for stearic acid, AA, and the cluster including TXB_2 were only nominally significant. This highlights the need to confirm these findings in an independent study population.

In summary, in this study, we measured a wide spectrum of FAs and oxylipins, and clustered the compounds based on their correlation structure to reduce the multi-collinearity in the regression analysis. The resulting clusters were consistent with shared dietary sources and endogenous metabolic pathways. The plasma concentration of the oxylipin, TXB_2, was inversely associated with risk of AMI. This association was unexpected, as TXB_2 is a marker for the pro-thrombotic TXA_2, and warrants further investigation in other prospective studies. Plasma long-chain n-3 PUFAs and stearic acid were associated with a lower risk of AMI and plasma total AA was associated with a higher risk of AMI. The nontypical findings for stearic acid and AA suggest that it is informative to examine FAs in non-Western populations with different dietary habits and that it is important to adjust for other FAs in the data analysis.

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