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THE ASSOCIATIONS OF C-REACTIVE PROTEIN WITH SERUM LEVELS OF POLYUNSATURATED FATTY ACIDS AND TRANS FATTY ACIDS AMONG MIDDLE-AGED MEN FROM THREE POPULATIONS

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Abstract: *Background:* C-reactive protein (CRP) and many fatty acids (FAs) have been linked to cardiovascular disease. Associations of serum CRP with FAs in different populations have not been established. *Methods:* Participants were 926 men aged 40-49 (2002-2006) from a population-based sample; 310 Whites from Pennsylvania, U.S., 313 Japanese from Shiga, Japan, and 303 Japanese Americans from Hawaii, U.S. Serum CRP (mg/L) was measured using immunosorbent assay while serum FAs (%) were measured using capillary-gas-liquid chromatography. *Results:* Whites had CRP (mg/L) levels higher than Japanese with Japanese Americans in-between (age-adjusted geometric mean "GM" 0.96, 0.38, 0.66, respectively). Whites had also higher levels of total n-6 FAs (%) and trans fatty acids (TFAs) but lower levels of marine-derived n-3 FAs compared to Japanese (41.78 vs. 35.05, 1.04 vs. 0.58, & 3.85 vs. 9.29, respectively). Japanese Americans had FAs levels in-between the other two populations. Whites had significant inverse trends between CRP and tertiles of total n-6 FAs (GM 1.20, 0.91 & 0.80; p=0.002) and marine-derived n-3 FAs (GM 1.22, 1.00 & 0.72; p<0.001) but a significant positive trend with TFAs (GM 0.80, 0.95 & 1.15; p=0.007). Japanese had a significant inverse trend between CRP and only total n-6 FAs (GM 0.50, 0.35 & 0.31; p<0.001). Japanese Americans had CRP associations with n-3 FAs, n-6 FAs, and TFAs similar to but weaker than Whites. *Conclusions:* With the exception of consistent inverse association of CRP with total n-6 FAs, there are considerable variations across the three populations in the associations of CRP with different FAs.

Key words: Epidemiology, c-reactive protein, polyunsaturated fatty acids, trans fatty acids, caucasian, japanese, japanese american.

Abbreviations: CRP: C-reactive protein; n-3 FAs: n-3 fatty acids; n-6 FAs: n-6 fatty acids; TFAs: trans fatty acids; LDL cholesterol: Low-density-lipoprotein cholesterol; HDL cholesterol: High-density-lipoprotein cholesterol.

Introduction

There is a growing understanding of the role of inflammation in atherosclerosis, coronary artery disease, and insulin resistance. C-reactive protein (CRP), a marker of inflammation, has been shown in multiple prospective studies to predict incident cardiovascular (CVD) events (1). The role of different FAs in inflammation and their associations with serum CRP have not been well-established. Moreover, it is unclear if such associations are similar in different populations having different levels of both CRP and FAs. Epidemiologic studies in White and Japanese populations showed that dietary intake of marine-derived n-3 fatty acids (n-3 FAs) is associated with lower serum level of CRP (2-5). However, such association was inconsistent in interventional studies (6-8). The association between CRP and n-6 fatty acids (n-6 FAs) remains inconsistent with both positive and negative associations being reported (8-10). Dietary intake of trans fatty acids (TFAs) has pro-inflammatory properties in Whites (11). No previous study reported the associations of CRP with different FAs levels in Japanese American.

Coronary heart disease (CHD) mortality is known to be lower in Japan than the US. CHD mortality in Japan is even decreasing over the last 3-4 decades despite the increased dietary fat intake accompanying westernization of life style (12, 13). Interestingly, Japanese who have low CRP levels (4), have higher intake of marine-derived n-3 FAs but lower intake of n-6 FAs and TFAs compared to Americans (14, 15). To study the associations of serum CRP with a wide range of serum levels of different FAs, in a population-based study, we examined 926 middle-aged US White, Japanese, and Japanese American men who were free of clinical CVD.

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Methods

I- Study population

The study population was described in detail elsewhere (16). During 2002-2006, a total of 926 men aged 40-49 years were randomly selected from three populations; 310 Whites from Pennsylvania, U.S., 313 Japanese from Shiga, Japan, 303 Japanese Americans from a representative sample of offspring of fathers who participated in the Honolulu Heart Program (17), Honolulu, Hawaii, US These offspring were the third or fourth generation of Japanese Americans without ethnic admixture. All participants were without clinical CVD disease, type 1 diabetes, or other severe diseases. Those who had missing CRP or FA data (N=9) as well as those with CRP>10 mg/L (N=8, to rule out the possibility of infections, trauma, and acute hospitalizations) were excluded from the current analysis. Our final sample was 909; 303 Whites, 312 Japanese in Japan, and 294 Japanese Americans. The study was approved by the Institutional Review Boards of University of Pittsburgh (Pittsburgh, US), Shiga University Medical Science (Otsu, Japan), and the Kuakini Medical Center (Honolulu, US). Informed consent was obtained from all participants before starting the study assessments.

II- Study assessments

The study protocol was described in detail elsewhere (16). Briefly, a self-administered questionnaire was used to obtain information on demography, smoking habits, alcohol drinking, medication use, and other factors. Blood pressure, weight and height were measured. In addition to CRP and FAs, fasting blood samples were used for centrally measuring serum low-density-lipoprotein (LDL) cholesterol, high-density-lipoprotein (HDL) cholesterol, triglycerides, glucose, insulin, and other markers (16). Serum CRP (mg/L) was measured using a calorimetric-competitive-enzyme-linked-immuno-sorbent assay. The sensitivity level of CRP was 0.16 mg/L. Those who had CRP levels below the minimum detected level were assigned 0.15 mg/L (n=128). They represented 28.2% of Japanese but 10.5% of Japanese American and 3.0% of White. The measurements of FAs were described in detail elsewhere (18). Briefly, serum FAs (%) were analyzed by capillary-gas-liquid chromatography (PerkinElmer Clarus 500) (18). Marine-derived n-3 FA were calculated as the sum of docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosapentaenoic acid (DPA, 22:5n-3); plant-derived n-3 FA as alpha-linolenic acid (18:3n-3) only; total n-6 FA as the sum of arachidonic acid (20:4n-6), linolenic acid (18:2n-6), gamma- linolenic acid (18:3n-6), dihomo-gamma-linolenic acid (20:3n-6), eicosadienoic acid (20:2n-6) and docosapentaenoic acid (22:5n-6); and TFAs as a sum of palmitelaidic acid (16:1t), trans-9 octadecaenoic acid (18:1t) and linolelaidic acid (18:2tt).

III-Statistical analyses

Between population differences in CRP and different FAs were examined using Kruskal-Wallis. Bonferroni test was used for all pairwise comparisons to adjust for multiple comparisons in log-transformed data. Population-specific associations of CRP with different FAs and potential CVD risk factors were assessed using Spearman's correlation coefficient. Due to skewness of CRP data in all populations, geometric rather than arithmetic mean was used. Age-adjusted and multivariate-adjusted geometric means (GMs) of CRP by population-

Table 1

Levels of serum C-reactive protein (CRP in mg/L) and serum fatty acids (%) as well as their Spearman correlations in White, Japanese, and Japanese American men

	White N=303			Japanese N=312			Japanese American N=294			Between-population p-value*
	Mean±SD	rho	P-value	Mean±SD	rho	P-value	Mean±SD	rho	P-value	
CRP	1.47±1.55	--	--	0.66±1.01	--	--	1.11±1.42	--	--	<0.001
Marine n-3 FA	3.85±1.73	-0.20	<0.001	9.29±2.96	0.02	0.697	4.84±2.19	-0.16	0.005	<0.001
Decosahexaenoic acid (DHA)	2.38±1.22	-0.18	0.002	5.92±1.65	0.01	0.854	3.16±1.34	-0.16	0.007	<0.001
Eicosapentaenoic acid (EPA)	0.79±0.57	-0.14	0.019	2.52±1.42	0.04	0.470	1.01±0.94	-0.12	0.037	<0.001
Plant n-3 FA	0.29±0.30	0.09	0.108	0.19±0.19	-0.11	0.050	0.43±0.41	-0.06	0.345	<0.001
Total n-6 FA	41.78±4.22	-0.21	<0.001	35.05±4.24	-0.19	0.001	41.61±4.32	-0.17	0.004	<0.001
Arachidonic acid	9.01±1.88	-0.06	0.302	6.58±1.31	-0.15	0.010	8.91±2.33	-0.21	<0.001	<0.001
Linoleic acid	29.84±4.11	-0.19	0.001	26.46±4.14	-0.16	0.006	30.01±4.27	-0.09	0.119	<0.001
Trans fatty acids (TFAs)	1.04±0.47	0.16	0.005	0.58±0.17	0.03	0.601	0.91±0.40	0.13	0.027	<0.001

*Additionally, all population pair-wise comparisons of CRP and FA levels were significantly different (p<0.01 for each) using Bonferroni test in log-transformed data, with the exception of total n-6 FA, arachidonic acid, and linoleic acid which were similar in White and Japanese American men

Table 2
Spearman correlation of C-reactive protein (CRP in mg/L) with cardiovascular risk factors in White, Japanese, and Japanese American men

	White		Japanese		Japanese American	
	rho*	P-value	rho*	P-value	rho*	P-value
Age (years)	0.003	0.959	0.03	0.619	-0.02	0.777
Systolic blood pressure (mmHg)	0.20	<0.001	0.11	0.059	0.19	0.001
Diastolic blood pressure (mmHg)	0.17	0.003	0.10	0.073	0.19	0.001
Body mass index (Kg/m2)	0.41	<0.001	0.28	<0.001	0.43	<0.001
LDL cholesterol (mg/dL)	0.01	0.831	0.11	0.046	0.00	0.945
HDL cholesterol (mg/dL)	-0.25	<0.001	-0.24	<0.001	-0.31	<0.001
Triglycerides (mg/dL)	0.25	<0.001	0.30	<0.001	0.25	<0.001
Fasting glucose (mg/dL)	0.09	0.108	0.05	0.371	0.20	0.001
Fasting insulin (μIU/mL)	0.30	<0.001	0.15	0.01	0.33	<0.001
Smoking (pack-years)	0.13	0.029	0.10	0.077	0.15	0.013
Ethanol intake (g/day)	-0.03	0.595	0.02	0.714	-0.21	<0.001
Lipid medications (%)	-0.03	0.586	-0.04	0.485	-0.06	0.343

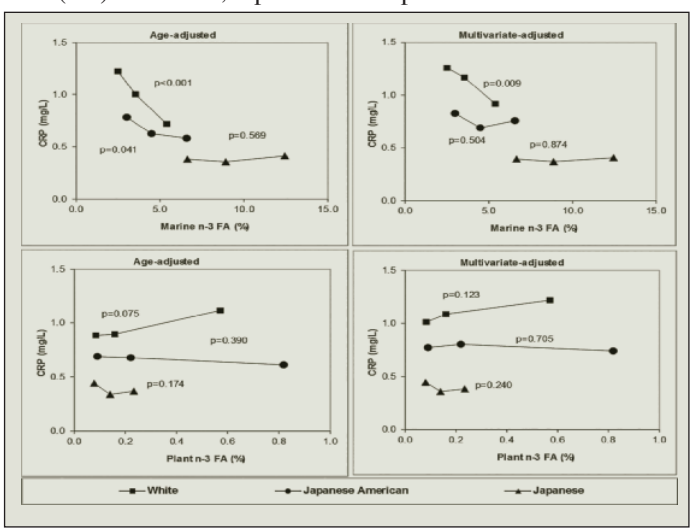
* Spearman's rank correlation coefficient. LDL, low density lipoprotein; and HDL, high density lipoprotein

specific tertiles of different FAs were calculated using linear regression models (model-1). The models were further adjusted for body mass index (BMI), current smoking (model-2), and ethanol intake (gm/day), lipid lowering medications use (model-3). GMs were calculated by estimating marginal means of logarithmic values of CRP then converting them back to a base 10 number. All P-values were two-tailed. P-value <0.05 was considered as significant. SPSS software (release 18.0, SPSS Inc., Chicago, U.S.) was used for all statistical analyses.

Results

Table 1 shows the levels (arithmetic mean ± standard deviation) of CRP (mg/L) and different FAs (%). CRP levels were higher in white men (1.47±1.55), lower in Japanese men (0.66±1.01), and intermediate in Japanese America men (1.11±1.42), the differences were significant (p<0.001 for all bi-racial comparisons). Similarly, TFAs were higher in White men (1.04±0.47), lower in Japanese men (0.58±0.17), and intermediate in Japanese American men (0.91±0.40, p<0.001 for each two-population comparisons). On the contrary, marine-derived n-3 FAs were lower in White men (3.85±1.73), higher in Japanese men (9.29±2.96), and intermediate in Japanese American men (4.84±2.19, p<0.001 for each two-population comparisons). Total n-6 FAs levels were similarly higher in White and Japanese American men (41.78±4.22 and 41.61±4.32, respectively) but lower in Japanese men (35.05±4.24, p<0.001 for both). Plant-derived n-3 FAs were higher in Japanese American men (0.43±0.41), lower in Japanese men (0.19±0.19), and intermediate in White men (0.29±0.30, p<0.001 for each two-population comparisons).

Figure 1
Age-adjusted and multivariate-adjusted geometric means of C-reactive protein (CRP) by population-specific tertiles of serum marine-derived n-3 and plant-derived n-3 fatty acids (FA) for White, Japanese and Japanese American men



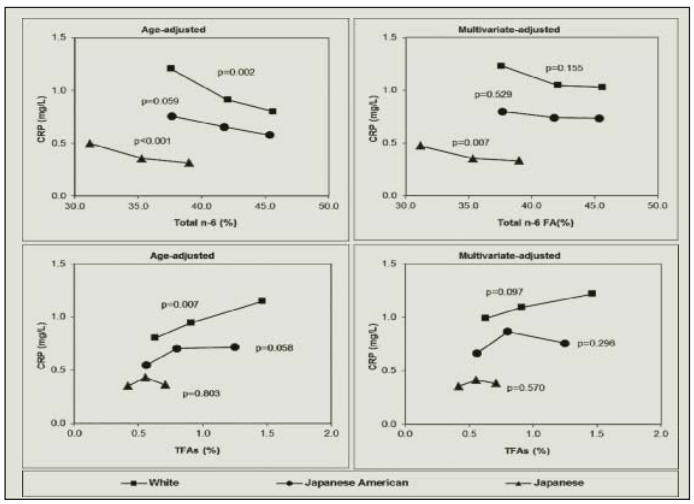
Multivariate-adjusted general linear models were adjusted for age, BMI, current smoking, ethanol intake, and lipid lowering medications. P-values were calculated for trends.

Table 1 shows the correlations of CRP with different FAs. CRP was inversely correlated with marine-derived n-3 FAs (including DHA and EPA) in both White and Japanese American men but not in Japanese men while CRP had (marginal) inverse correlation with plant-derived n-3 FAs only in Japanese men. CRP was inversely correlated with total n-6

FAs levels in all populations. CRP was positively correlated with TFAs in White and Japanese American men. CRP levels were associated with a worse profile for many CVD factors in all populations (Table 2). For example, CRP levels in all populations were positively correlated with BMI, triglycerides, and insulin and negatively correlated with HDL cholesterol. Additionally, CRP levels were positively correlated with blood pressure and pack-years of smoking in White and Japanese American men.

Figure 2

Age-adjusted and multivariate-adjusted geometric means of C-reactive protein (CRP) by population-specific tertiles of serum total n-6 fatty acids (FA) and trans fatty acids (TFAs) for White, Japanese and Japanese American men



Multivariate-adjusted general linear models were adjusted for age, BMI, current smoking, ethanol intake, and lipid lowering medications. P-values were calculated for trends.

Figures 1 and 2 show age-adjusted and multivariate-adjusted GMs of CRP by tertiles of different FAs. GMs (± standard errors) of CRP were 0.96±1.06, 0.38±1.06, and 0.66±1.06 in White, Japanese, and Japanese American men, respectively. Significant inverse trends between CRP and marine-derived n-3 FAs were observed in White men in age adjusted (GMs of CRP were 1.22, 1.00 & 0.72; p<0.001) as well as in multivariate-adjusted models (models 2 & 3). Such inverse trends were not observed in Japanese men and were observed only in age-adjusted model in Japanese American men (GMs of CRP were 0.78, 0.63 & 0.58; p=0.041) (Figure 1). In White men only, a marginally significant positive trend between CRP and plant-derived n-3 FAs was observed in age adjusted (p=0.075) but not in multivariate-adjusted models (Figure 1). In age-adjusted models, there were inverse trends between CRP and total n-6 FAs in White (GMs of CRP were 1.20, 0.91 & 0.80; p=0.002), Japanese (GMs of CRP were 0.50, 0.35 & 0.31; p<0.001), and Japanese American men (GMs of CRP were 0.76, 0.65 & 0.58; p=0.059) (Figure 2). Such inverse trends were attenuated and became non-significant in White (p=0.177) and Japanese American men (p=0.880) after adjusting for BMI and current smoking (model-2), but remained significant in Japanese (p=0.007). Positive trends between CRP and TFAs were observed only in age-adjusted models in White (GMs of CRP were 0.80, 0.95 & 1.15; p=0.007) and Japanese American men (GMs of CRP were 0.55, 0.70 & 0.72; p=0.058) (Figure 2).

Discussion

The current study is unique in examining the associations between serum CRP and serum levels of different FAs in a population-based sample of middle age men free of clinical CVD from three populations. Although attenuated or disappeared after adjusting for differences in CVD risk factors, CRP had inverse association with total n-6 FAs in all populations. In White men, this inverse association was seen with linoleic acid but not arachidonic acid. Interestingly, a meta-analysis by Harris and colleagues showed that linoleic acid but not arachidonic acid has inverse association with cardiovascular events (19). There is increasing evidence that linoleic acid, a major component of n-6 FAs, has anti-inflammatory properties being associated with lower CRP in both White (20, 21) and Japanese (22). Moreover, a recent multi-ethnic report including White showed that linoleic acid is inversely proportional with CRP irrespective of race (23). Although several arachidonic acid-derived eicosanoids may have a pro-inflammatory role, it has been suggested that several arachidonic acid-derived eicosanoids may play an anti-inflammatory role (10).

Although attenuated or disappeared after adjusting for differences in CVD risk factors, CRP had an inverse association with marine-derived n-3 FAs in in both White and Japanese American men but not in Japanese men. Supporting our findings in White men, many epidemiologic studies in Whites showed that marine-derived n-3 FAs have an inverse association with CRP and pro-inflammatory cytokines (3, 20, 23). However, results of randomized clinical trial of marine-derived n-3 FAs on CRP or other pro-inflammatory cytokines were inconsistent (7, 8). The anti-inflammatory effect of the n-3 FAs is believed to be due to reducing the levels of inflammatory eicosanoids (prostaglandins and the leukotrienes), cytokines, and reactive oxygen species and the expression of adhesion molecules. Experimental studies show that n-3 FAs act both directly by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism, and indirectly by altering the expression of inflammatory genes through effects on transcription factor activation (24).

CRP in Japanese was reported to be inversely associated with the dietary intake of marine-derived n-3 FAs in most (4, 5) but not all studies (25). The fact that we did not observe such significant association in Japanese is partly due to the small sample size and low risk of our subjects. The significant inverse association of CRP with the dietary intake of marine-derived

n-3 FAs among Japanese was typically observed in very large cross-sectional studies (>10,000) or in a high risk group (e.g. smokers) (4, 5). Alternatively, Yoneyama et al. reported that dietary intake of EPA and DHA does not have a significant association with CRP (9). We found that those who had CRP level below the minimum detected level (<0.16 mg/L, n=128) were 28.2% of Japanese whereas 10.5% of Japanese American and 3.0% of White. The finding may be in accordance with recent studies in Japan reporting that a cutoff point of CRP in predicting CHD and stroke is 1.0 mg/L (26, 27), which is much lower than that in the US (3.0 mg/L).

The association between CRP and plant-derived n-3 FAs was weak and inconsistent; positive in White men and negative in other populations. Although plant-derived n-3 FAs is elongated to EPA, the conversion rate is very low (0.2%) (28). Additionally, a recent meta-analysis showed that increased consumption of marine-derived n-3 but not plant-derived n-3 FAs reduces coronary death and sudden cardiac death (29). CRP associations with different FAs in Japanese American men in this study were closer to White men than Japanese men. There are generally lack of data evaluating the associations of CRP and different FAs levels in Japanese American.

In accordance with our finding, TFAs have pro-inflammatory properties in Whites (11). The pro-inflammatory properties of TFAs were suggested to partially explain the effect of TFAs on the CVD risk beyond their traditional effects on blood lipids (30). The mechanisms underlying these pro-inflammatory effects are not well-established, but may involve TFAs incorporation into endothelial cell, monocyte/macrophage, or adipocyte cell membranes (affecting membrane signaling pathway relating to inflammation) or ligand-dependent effects on peroxisome proliferator-activated receptor or retinoid X receptor pathways (31).

In the current study, we used serum FAs rather than dietary intake of FAs to study their association with CRP. A number of epidemiologic studies used serum or other tissue FAs levels to study the anti-inflammatory properties of FAs (2, 20, 21). The levels of n-3 and n-6 FA measured in blood or other tissues were reported to be a good indicator of FAs intake (32, 33) in addition to avoiding the measurement errors associated with dietary assessment. As expected, White men had lower levels of marine-derived n-3 FAs and higher levels of total n-6 FAs and TFAs. Consistent with these findings, the International study of macro- and micro-nutrients and blood pressure (INTERMAP) study showed that American diet has less marine-derived n-3 FAs and more n-6 FAs and TFAs, compared to the Japanese diet (14, 15). The Japanese ratio of n-3 to n-6 in the current study was three-folds higher than Whites and more than two-folds higher than Japanese Americans. The dietary ratio of n-3 to n-6 is much lower in the western (1 to 20) compared to the Japanese diet (1 to 4-5) (9) as the dietary intake of polyunsaturated FAs is primarily n-6 FAs in the former but marine-derived n-3 FAs in the later. Japanese American, who adopted western life style, were reported to have lower intake of marine-derived n-3 FA but higher intake of n-6 FAs compared to Japanese (34).

Our study had strengths and limitations. A major strength of the current study was studying the associations of CRP and different FAs levels in a three-population sample across a wide range of levels of both CRP and different FAs levels probably reflecting significant dietary differences. In addition, using serum FAs levels which are believed to be a good indicator of dietary intake of FAs, allowing us to study such associations in a physiologic setting without the measurement errors associated with dietary assessment. The cross-sectional design of the current study precluded any causal associations. In addition, the study results should not be generalized beyond middle aged men. Although we had many race-specific associations, a post hoc power analysis showed enough sample size relatively small differences.

In conclusion, with the exception of consistent inverse association of CRP with total n-6 FAs, there are considerable variations across the three populations in the associations of CRP with different FAs. Significant associations are typically attenuated or disappeared after adjusting for between-population differences in CVD risk factors.

Conflict of Interest Disclosures: Corresponding author and all coauthors have nothing to disclose.

Ethical standards: The study was approved by the Institutional Review Boards of University of Pittsburgh (Pittsburgh, US), Shiga University Medical Science (Otsu, Japan), and the Kuakini Medical Center (Honolulu, US). Informed consent was obtained from all participants before starting the study assessments.

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