Circulating Levels of 8 Cytokines and Marine n-3 Fatty Acids and Indices of Obesity in Japanese, White, and Japanese American Middle-Aged Men

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This study examines the differences in circulating levels of cytokines among Japanese in Japan (JJ), Japanese Americans (JA), and whites and their associations with obesity and marine n-3 fatty acids (FA) in a cross-sectional population-based study of 297 men aged 40–49 (100 JJ, 99 whites, and 98 JA). Experimental studies show that cytokines are associated with obesity positively and marine n-3 FA inversely. Serum interleukin-1 α (IL-1 α), IL-1 receptor agonist (IL-1ra), IL-4, IL-8, IL-10, inducible protein-10 (IP-10), tumor necrosis factor- α (TNF- α), monocyte chemoattractant protein-1 (MCP-1), and marine n-3 FA were determined. Body mass index (BMI), waist circumference, and computed tomography-measured visceral and subcutaneous adipose tissues were determined. The JJ had significantly lower levels of IL-1 α , IL-4, IL-8, MCP-1, and TNF- α than whites and JA. Whites and JA had similar levels of IL-1 α , IL-4, and IL-8 whereas whites had significantly higher levels of MCP-1 and TNF- α than JA. The JJ were least obese (BMI (kg/m²), mean $\pm$ standard deviation) 23.6 ± 2.8 , 27.9 ± 4.6 , and 27.9 ± 4.5 for JJ, whites, and JA, respectively. The JJ had marine n-3 FA about 100% higher than whites and JA (serum marine n-3 FA (%), median (interquartile range) 8.79 (7.41, 11.16), 3.47 (2.63, 4.83), and 4.44 (3.33, 6.01) for JJ, whites, and JA, respectively). Generally cytokines had weak and nonsignificant associations with indices of obesity and nonsignificant associations with marine n-3 FA. BMI had significant inverse associations with IL-1 α , IL-4, and IL-8 in JA ($P < 0.05$). Marine n-3 FA had marginally significant inverse associations with IL-8 in JJ ($P = 0.055$) and TNF- α in whites ($P = 0.076$). The JJ had lower levels of many cytokines than whites and JA. Generally cytokines had weak and nonsignificant associations with indices of obesity and marine n-3 FA. Further investigation is needed to determine why JJ had lower circulating levels of cytokines.

Introduction

CYTOKINES ARE A GROUP of molecules that mediate intercellular communication at very low concentrations (Cohen 2004; Feldmann 2008). Abnormality in cytokines and their regulations are related to many diseases, for example, cancer (Dranoff 2004), cardiovascular disease (Zernecke and others 2008), chronic obstructive pulmonary disease (Barnes 2008), and autoimmune disease (Skurkovich and Skurkovich

2007). Thus inhibitors of cytokine production and action have been widely investigated as therapeutic agents in many diseases (Feldmann 2008). Multiplex proteomics is a novel technology that enables the measurement of multiple protein analytes including cytokines from the same sample (Knowles and others 2003).

Obesity is recognized as a low-grade inflammatory state resulting from secretion of proinflammatory cytokines

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from the adipose tissue, for example, interleukin (IL)-6, tumor necrosis factor- α (TNF- α), and monocyte chemoattractant protein-1 (MCP-1). Clinical studies comparing obese with non-obese volunteers show that some cytokines, that is, IL-1 receptor agonist (IL-1ra), IL-8, IL-10, MCP-1, and TNF- α , are associated with obesity (Meier and others 2002; Straczkowski and others 2002; Bruun and others 2003; Esposito and others 2003; Olszanecka-Glinianowicz and others 2004; Christiansen and others 2005; Park and others 2005; Kim and others 2006; Ruotsalainen and others 2006). Only a few previous population studies, however, have examined the associations of multiple cytokines with obesity (Herder and others 2006, 2007; Wong and others 2008). Experimental studies show that secretion of some cytokines is different between visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). VAT secretes IL-8 and MCP-1 more than SAT (Bruun and others 2004, 2005). No previous population study examined the association of multiple cytokines with VAT and SAT.

Marine n-3 fatty acids (FA) have anti-inflammatory effects. Experimental studies show that marine n-3 FA decrease the generation of inflammatory cytokines, for example, TNF- α , IL-6, and IL-8 (Calder 2006). Very few population studies, however, have examined the association of multiple cytokines with serum marine n-3 FA (Ferrucci and others 2006).

We have previously reported that among men in the post World War II birth cohort, that is, men aged 40–49, the Japanese in Japan (JJ) had much lower levels of body mass index (BMI) and C-reactive protein (CRP) than whites in the United States (US) and Japanese Americans (JA) whereas whites and JA had similar levels of BMI and CRP. Additionally, the JJ had about 2-fold higher levels of serum marine n-3 FA compared with whites and JA (Sekikawa and others 2008).

In this study, we hypothesized that circulating levels of cytokines are lower in the JJ compared with whites and JA. Then we examined the associations of circulating levels of cytokines with indices of obesity including VAT and SAT as well as serum marine n-3 FA. To test the hypothesis and examine the associations, we measured interleukin-1 α (IL-1 α), IL-1ra, IL-4, IL-8, IL-10, TNF- α , inducible protein-10 (IP-10), and MCP-1 in samples from EBCT and Risk Factor Assessment among Japanese and US Men in the Post World War II Birth Cohort (ERA JUMP), a population-based cross-sectional study of JJ, white, and JA men aged 40–49 (Sekikawa and others 2008).

Materials and Methods

Study population

We randomly selected 300 men aged 40–49 from the ERA JUMP Study: 100 JJ, 100 whites in the United States, and 100 JA. The recruitment of the ERA JUMP Study was previously described in detail (Sekikawa and others 2008). In brief, during 2002–2006, 926 men aged 40–49 were randomly selected: 313 JJ from Kusatsu, Shiga, Japan; 310 whites from Allegheny County, PA; and 303 JA from a representative sample of offspring of fathers who participated in the Honolulu Heart Program Honolulu, HI (Kagan and others 1974). These offspring were the third or fourth generation JA without ethnic admixture. All participants were without clinical cardiovascular disease, type 1 diabetes, or other severe diseases.

Among these 300 men, we excluded 3 men with missing data. Our final sample was 100 JJ, 99 whites, and 98 JA. Informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of Shiga University of Medical Science, Otsu, Japan, University of Pittsburgh, Pittsburgh, PA and Kuakini Medical Center, Honolulu, HI.

Clinical and laboratory measurements

All participants underwent a physical examination, life-style questionnaire, and laboratory assessment as described previously (Sekikawa and others 2008). Body weight and height were measured while the participant was wearing light clothing without shoes. BMI was calculated as weight (kg)/height squared (m²). Waist circumference (WC) was measured at the level of the umbilicus while the participant was standing erect.

A self-administered questionnaire was used to obtain information on demography, smoking habits, alcohol drinking, and other factors. Alcohol drinkers were defined as those who drank alcohol ≥ 2 days per week. Hypertension was defined as systolic blood pressure (BP) ≥ 140 mmHg, diastolic BP ≥ 90 mmHg, or use of antihypertensive medications. Diabetes mellitus was defined as fasting serum glucose level ≥ 7 mmol/L or use of anti-diabetic medications.

Serum samples were stored at -80°C , shipped on dry ice to University of Pittsburgh, and determined for levels of LDL-cholesterol, HDL-cholesterol, triglycerides, glucose, insulin, and CRP. The sensitivity level of CRP was 0.16 mg/L. Serum FA were determined by capillary gas-liquid chromatography (Sekikawa and others 2008). Serum marine n-3 FA were defined as the sum of eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids.

To determine serum levels of IL-1 α , IL-1ra, IL-4, IL-8, IL-10, IP-10, MCP-1, and TNF- α , multianalyte profiling was performed on the Bio-Plex Luminex system (Bio-Rad Laboratories, Inc., Hercules, CA) at the University of Vermont, using a Millipore cytokine 8-plex panel (Linco Research, St. Charles, MO). Acquired fluorescence data were analyzed by Bio-Plex Manager software (version 4.1; Bio-Rad Laboratories). The sensitivity levels of cytokine assays were 1.99, 2.99, 1.99, 0.63, 0.63, and 0.79 pg/mL for IL-1 α , IL-1ra, IL-4, IL-8, IL-10, and TNF- α , respectively. The intra-assay coefficients of variations examined in our samples were 11.2%, 10.1%, 13.5%, 8.4%, 14.3%, 13.7%, 9.9%, and 7.1% for IL-1 α , IL-1ra, IL-4, IL-8, IL-10, IP-10, MCP-1, and TNF- α , respectively.

VAT and SAT

VAT and SAT were evaluated at the level between the fourth and fifth lumbar vertebrae using a GE-Imatron C150 EBT scanner (GE Medical Systems, South San Francisco, CA) at all 3 centers (Kadowaki and others 2006). All the images were read at the Cardiovascular Institute, University of Pittsburgh Medical Center by a trained reader blind to study center and participant's characteristics. Using a trace function, a region of interest line was drawn at the junction of the subcutaneous fat and the abdominal wall musculature, extending around the body to the back muscles. Using a pixel range of -190 to -30 Hounsfield unit as the range for fat, the area of adipose tissue within the region of interest was

determined. The fat within this circle was considered to be VAT. The fat area for the entire image was then determined and the difference in fat area between the whole image and VAT was equal to SAT. Intra-class correlation coefficients at our reading center are 0.99 for both VAT and SAT.

Statistical analysis

To compare continuous variables among samples, ANOVA or the Kruskal–Wallis test was used as appropriately. To compare categorical variables, the Mantel–Haenszel test was used. To assess correlations between each cytokine and each index of obesity, Spearman's rho was used. When levels of cytokines were below the sensitivity levels, we inserted a value 0.1 pg/mL lower than the sensitivity level. To examine associations of serum marine n-3 FA with each cytokine, we made a tertile group of marine n-3 FA for each of the JJ, whites, and JA, then compared the cytokine levels across tertile groups. To compare levels of cytokines by smoking status (current smoker versus non-current smoker) in each population, the Mann–Whitney *U*-test was used. As we were testing specific hypotheses regarding the influence of obesity, marine n-3 FA, and other factors on cytokines, adjustments for multiple comparisons were not made in accordance with recent recommendations (Perneger 1998). Although type I error may exist in our exploratory analyses, biological plausibility of our hypotheses minimizes the potential for type I error. All *P* values were 2-tailed. *P* value < 0.05 was considered as significant. SPSS (16.0 for Windows, Chicago, IL) was used for all statistical analyses.

Results

The JJ were least obese among the samples from the 3 cohorts as measured by BMI, WC, VAT, and SAT (Table 1). Whites and JA were similarly obese. The JJ had the lowest levels of insulin and CRP whereas whites had similar insulin or higher CRP levels compared with the JA. The JJ had levels of serum marine n-3 FA about 2-fold as high as those in whites and JA.

Generally, the JJ had the lowest circulating levels of cytokines among the samples from the 3 cohorts whereas whites had similar or higher levels compared with JA (Table 2). This held true for IL-1 α , IL-4, IL-8, MCP-1, and TNF- α . The distributions of IL-1 α , IL-1ra, IL-4, IL-8, and IL-10 were much skewed to the right and 22.2% to 85.0% were under the detection level (Table 2). Meanwhile, the JJ had the highest levels of IP-10 and had similar levels of IL-10 among the 3 samples.

Circulating levels of cytokines generally had weak and nonsignificant correlations with indices of obesity (Table 3). Positive significant correlations were found in IP-10 with VAT and SAT in the JJ, IL-1ra with VAT and SAT in whites, and IP-10 with BMI, WC, VAT, and SAT in the JA. Additionally, in the JA inverse significant correlations were found in IL-1 α with BMI, WC, VAT, and SAT, IL-4 with BMI and WC, and IL-8 with BMI and WC.

Circulating levels of cytokines had weak and nonsignificant associations with serum marine n-3 FA in each of the 3 samples (Table 4). Inverse and marginally significant associations were found in IL-8 in the JJ (*P* = 0.055) and TNF- α in whites (*P* = 0.076).

TABLE 1. BASIC CHARACTERISTICS OF JAPANESE, WHITE, AND JAPANESE AMERICAN MEN AGED 40–49

	Japanese in Japan (<i>n</i> = 100)	Whites (<i>n</i> = 99)	Japanese Americans (<i>n</i> = 98)	<i>P</i>
Age (years)	45.1 $\pm$ 2.8	45.4 $\pm$ 2.7	46.0 $\pm$ 3.0	
BMI (kg/m ²)	23.6 $\pm$ 2.8	27.9 $\pm$ 4.6	27.9 $\pm$ 4.5	¶§
Waist circumference (cm)	84.6 $\pm$ 7.3	98.4 $\pm$ 11.9	93.9 $\pm$ 11.5	¶†§
VAT (cm ²)	77.7 $\pm$ 29.1	99.2 $\pm$ 44.3	104.1 $\pm$ 52.4	¶†
SAT (cm ²)	79.6 $\pm$ 30.3	157.2 $\pm$ 76.0	141.9 $\pm$ 66.2	¶†
Systolic blood pressure (mmHg)	124.7 $\pm$ 14.9	121.4 $\pm$ 10.3	127.2 $\pm$ 13.2	¶†
Hypertension (%)	22.0	13.1	32.7	†
LDL-cholesterol (mmol/L)	3.47 $\pm$ 0.99	3.46 $\pm$ 0.89	3.14 $\pm$ 0.96	§
Triglycerides (mmol/L)	1.74 $\pm$ 1.01	1.78 $\pm$ 1.59	2.36 $\pm$ 1.98	
HDL-cholesterol (mmol/L)	1.39 $\pm$ 0.38	1.23 $\pm$ 0.31	1.31 $\pm$ 0.30	¶
Glucose (mmol/L)	5.97 $\pm$ 1.07	5.59 $\pm$ 0.57	6.21 $\pm$ 1.16	¶†
Insulin (pmol/L)	68.1 $\pm$ 29.1	100.7 $\pm$ 57.6	105.6 $\pm$ 76.4	¶§
C-Reactive protein (mg/L)	0.32 (0.17, 0.78)	0.85 (0.48, 1.73)	0.68 (0.34, 1.17)	¶†§
Serum marine n-3 fatty acids (%)	8.79 (7.41, 11.16)	3.47 (2.63, 4.83)	4.44 (3.33, 6.01)	
Type 2 diabetes mellitus (%)	8.0	3.0	17.3	†
Current cigarette smoker (%)	54.0	7.1	15.3	¶§
Alcohol drinker (%)	62.0	50.5	31.6	†§

Values are means (standard deviations) unless stated otherwise.

Hypertension was defined as systolic blood pressure $\geq$ 140 mmHg, diastolic blood pressure $\geq$ 90 mmHg, or hypertensive medication. Diabetes was defined as fasting glucose $\geq$ 126 mg/dL or diabetes medication. Alcohol drinker was defined as those who drank alcohol 2 days per week or more.

Abbreviations: VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

¶*P* < 0.05 between the Japanese in Japan versus whites.

†*P* < 0.05 between whites and Japanese Americans.

§*P* < 0.05 between the Japanese in Japan versus Japanese Americans.

TABLE 2. DISTRIBUTIONS OF CYTOKINES IN JAPANESE, WHITE, AND JAPANESE AMERICAN MEN AGED 40–49

	Median and interquartile range			% not detected		
	Japanese in Japan (n = 100)	Whites (n = 99)	Japanese Americans (n = 98)	Japanese in Japan (n = 100)	Whites (n = 99)	Japanese Americans (n = 98)
IL-1 α (pg/mL)	nd (nd, nd)	77 (nd, 617)	98 (nd, 834) ^{‡§}	85.0	36.4	41.8 ^{‡§}
IL-1ra (pg/mL)	22 (nd, 89)	49 (9, 119)	8 (nd, 80) ^{‡†}	39.0	22.2	47.9 ^{‡†}
IL-4 (pg/mL)	nd (nd, 23)	101 (nd, 925)	180 (nd, 1,100) ^{‡§}	72.0	33.3	32.7 ^{‡§}
IL-8 (pg/mL)	nd (nd, 4.0)	10.0 (0.8, 48.4)	11.1 (nd, 50.7) ^{‡§}	62.0	24.2	32.7 ^{‡§}
IL-10 (pg/mL)	2.2 (nd, 9.6)	7.1 (nd, 13.2)	4.3 (nd, 17.4)	36.0	25.3	36.0
IP-10 (pg/mL)	253 (197, 319)	199 (147, 278)	168 (127, 247) ^{‡†§}	0	0	0
MCP-1 (pg/mL)	146 (108, 177)	274 (226, 322)	157 (126, 197) ^{‡†}	0	0	0
TNF- α (pg/mL)	2.1 (nd, 4.1)	4.4 (2.9, 6.7)	3.5 (2.4, 4.9) ^{‡†§}	29.0	3.0	7.1 ^{‡§}

IL-1 α : Interleukin-1 α ; IL-1ra: interleukin-1 receptor agonist; IL-4: interleukin-4; IL-8: interleukin-8; IL-10: interleukin-10; IP-10: interferon-inducible protein-10; MCP-1: monocyte chemoattractant protein-1; TNF- α : tumor necrosis factor- α ; nd: not detected.

[‡] $P < 0.05$ between the Japanese in Japan versus whites.

[†] $P < 0.05$ between whites and Japanese Americans.

[§] $P < 0.05$ between the Japanese in Japan versus Japanese Americans.

We examined Spearman's correlations among cytokines. All 3 samples had strong correlations between IL-4 and IL-8, between IL-4 and IL-1 α , and between IL-1 α and IL-8 (all $P < 0.01$). These correlations were very strong in whites and JA (Spearman's rho > 0.9). CRP had significant positive correlations with IL-1ra in the JJ and whites (Spearman's rho 0.258, $P < 0.01$ and 0.218, $P < 0.05$, respectively), with IP-10 in whites and JA (Spearman's rho 0.207, $P < 0.05$ and 0.238, $P < 0.05$, respectively), with MCP-1 in whites (Spearman's rho 0.408, $P < 0.01$), and with TNF- α in whites (Spearman's rho 0.310, $P < 0.01$). Other than these, the majority of the correlations among these cytokines and CRP were weak and not statistically significant.

Current smokers tended to have higher levels of TNF- α in each sample although the difference did not reach statistical significance. Current smokers had significantly higher levels of IL-1ra in the JJ. In contrast, current smokers had significantly lower levels of IL-1 α in the JJ and JA as well as IL-4 and IL-8 in JA (data not shown). Alcohol drinking status had no significant association with cytokines in any of the 3 samples (data not shown).

Discussion

This study has shown that circulating levels of many cytokines were lower in the JJ compared with whites and JA. The study has also shown that correlations of these cytokines with indices of obesity including VAT and SAT as well as serum marine n-3 FA were weak and mostly not statistically significant. Thus it appears to be unlikely that the differences in obesity and marine n-3 FA between the JJ and the other 2 samples accounts for the low circulating levels of cytokines observed in the JJ in men aged 40–49. To our best knowledge, this is the first study to compare circulating levels of multiple cytokines among different population samples and to examine their associations with indices of obesity including VAT and SAT as well as marine n-3 FA.

Our observation that IL-1ra, IL-10, MCP-1, and TNF- α had weak correlations with BMI and WC in all samples from the 3 cohorts is inconsistent with the results from previous clinical studies reporting the significant positive associations of these cytokines with obesity (Meier and others 2002; Straczkowski and others 2002; Bruun and others 2003;

TABLE 3. SPEARMAN CORRELATION COEFFICIENT (RHO) BETWEEN INDICES OF OBESITY (BODY MASS INDEX, WAIST CIRCUMFERENCE, VISCERAL ADIPOSE TISSUE, AND SUBCUTANEOUS ADIPOSE TISSUE) AND CYTOKINES IN JAPANESE, WHITE, AND JAPANESE AMERICAN MEN AGED 40–49

	Japanese in Japan (n = 100)				Whites (n = 99)				Japanese Americans (n = 98)			
	BMI	WC	VAT	SAT	BMI	WC	VAT	SAT	BMI	WC	VAT	SAT
IL-1 α	0.064	−0.088	−0.054	−0.060	−0.114	−0.091	−0.001	−0.044	−0.253*	−0.256*	−0.178	−0.224*
IL-1ra	0.170	0.107	0.111	0.068	0.160	0.193	0.207*	0.261**	0.066	0.156	0.171	0.119
IL-4	−0.047	−0.157	−0.070	−0.118	−0.090	−0.048	0.038	0.014	−0.243*	−0.225*	−0.160	−0.199
IL-8	−0.004	−0.130	−0.018	−0.046	−0.106	−0.089	0.012	−0.024	−0.226*	−0.212*	−0.131	−0.193
IL-10	−0.177	−0.094	−0.108	−0.101	0.123	0.060	0.030	0.168	−0.090	−0.038	0.006	−0.085
IP-10	0.191	0.164	0.232*	0.199*	0.133	0.152	0.106	0.124	0.407**	0.331**	0.330**	0.297**
MCP-1	−0.033	−0.007	0.079	0.045	0.087	0.192	0.195	0.130	−0.049	−0.067	0.077	−0.099
TNF- α	0.075	0.070	0.048	0.134	0.076	0.160	0.115	0.148	0.139	0.155	0.137	0.101

BMI: Body mass index; WC: waist circumference; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue; IL-1 α : interleukin-1 α ; IL-1ra: interleukin-1 receptor agonist; IL-4: interleukin-4; IL-8: interleukin-8; IL-10: interleukin-10; IP-10: interferon-inducible protein-10; MCP-1: monocyte chemoattractant protein-1; TNF- α : tumor necrosis factor- α .

* $P < 0.05$; ** $P < 0.01$.

TABLE 4. ASSOCIATIONS OF CYTOKINES WITH MARINE N-3 FATTY ACIDS IN JAPANESE, WHITE, AND JAPANESE AMERICAN MEN AGED 40-49

Serum marine n-3 fatty acids tertile	Japanese in Japan (n = 100)				Whites (n = 99)				Japanese Americans (n = 98)			
	Low	Middle	High	P for trend	Low	Middle	High	P for trend	Low	Middle	High	P for trend
IL-1 α (pg/mL)	nd (nd, nd)	nd (nd, nd)	nd (nd, nd)	0.514	123 (nd, 1,259)	69 (nd, 327)	77 (nd, 505)	0.333	68 (nd, 1,185)	nd (nd, 352)	364 (nd, 1,173)	0.532
IL-1 α (pg/mL)	40 (nd, 118)	18 (nd, 53)	19 (nd, 86)	0.437	60 (7, 135)	38 (9, 102)	58 (9, 120)	0.931	20 (nd, 97)	nd (nd, 65)	10 (nd, 74)	0.506
IL-4 (pg/mL)	nd (nd, 77)	nd (nd, 5)	nd (nd, 18)	0.459	141 (13, 1,530)	75 (nd, 585)	101 (nd, 955)	0.261	221 (nd, 1,486)	32 (nd, 463)	349 (nd, 1,295)	0.725
IL-8 (pg/mL)	1.3 (nd, 6.2)	nd (nd, 1.8)	nd (nd, 1.2)	0.055	13.0 (4.2, 77.1)	8.6 (nd, 31.8)	10.0 (1.5, 48.7)	0.348	10.2 (1.2, 79.9)	2.6 (nd, 25.0)	36.4 (nd, 78.8)	0.633
IL-10 (pg/mL)	0.8 (nd, 12.0)	5.0 (0.7, 13.0)	5.5 (nd, 9.3)	0.344	7.1 (nd, 12.8)	4.6 (nd, 12.2)	8.1 (1.5, 17.5)	0.498	6.1 (1.2, 22.3)	nd (nd, 9.5)	6.4 (nd, 24.2)	0.649
IP-10 (pg/mL)	242 (183, 313)	248 (195, 318)	265 (209, 329)	0.331	192 (140, 259)	194 (141, 232)	223 (157, 314)	0.413	183 (141, 300)	157 (118, 239)	161 (121, 213)	0.147
MCP-1 (pg/mL)	144 (102, 199)	134 (110, 167)	150 (121, 170)	0.610	282 (240, 330)	279 (205, 325)	270 (229, 295)	0.231	162 (128, 162)	149 (121, 149)	155 (118, 200)	0.618
TNF- α (pg/mL)	2.1 (nd, 4.3)	1.9 (1.1, 3.7)	2.2 (nd, 4.1)	0.732	4.9 (3.6, 5.5)	4.9 (2.8, 7.5)	3.1 (2.5, 6.4)	0.076	4.2 (2.7, 6.1)	3.2 (2.0, 4.1)	3.7 (2.4, 4.8)	0.328

Data are expressed as median (interquartile range).

nd: not detected; IL-1 α : interleukin-1 α ; IL-1 β : interleukin-1 β ; IL-4: interleukin-4; IL-8: interleukin-8; IL-10: interleukin-10; IP-10: interferon-inducible protein-10; MCP-1: monocyte chemoattractant protein-1; TNF- α : tumor necrosis factor- α .

Esposito and others 2003; Olszanecka-Glinianowicz and others 2004; Christiansen and others 2005; Park and others 2005; Kim and others 2006; Ruotsalainen and others 2006). Typically, these clinical studies compared circulating levels of cytokines between a small number of nonobese and extreme obese subjects. Thus population studies like the current study are expected to observe weak associations of these cytokines with obesity. In fact, a population study in 290 women reported no significant association of MCP-1 or TNF- α with WC (Wong and others 2008). Likewise, a population study in 519 adolescents reported no significant association of MCP-1 or TNF- α with BMI or WC (Herder and others 2007). Additionally, a population study of 722 men and women reported no significant association of MCP-1 with BMI (Herder and others 2006).

We found that IL-1 α , IL-4, and IL-8 had significant inverse correlations with BMI and WC in JA but not in the JJ or whites. Although clinical studies reported a positive significant association of IL-8 with obesity (Strackowski and others 2002; Bruun and others 2003; Kim and others 2006), results from population studies are inconsistent. A population study in 519 adolescents reported a significant inverse association of IL-8 with WC (Herder and others 2007) whereas a population study of 722 men and women reported a significant positive association of IL-8 with BMI (Herder and others 2005). Meanwhile, a population study in 290 women reported no significant association of IL-1 α , IL-4, or IL-8 with WC (Wong and others 2008).

We found that correlations between circulating levels of cytokines and CT-measured VAT or SAT were weak and mostly nonsignificant. Exceptions were IL-1 α in JA, IL-1ra in whites, and IP-10 in the JJ and JA. Correlation coefficients of each cytokines, however, were not much different between VAT and SAT. Although experimental studies show that some cytokines are secreted more from VAT than SAT whereas other cytokines are similarly secreted from both VAT and SAT (Yang and Smith 2007), circulating levels of cytokines may not reflect local pathophysiology of cytokines.

Our observation of a marginal significant inverse association of marine n-3 FA with IL-8 in the JJ, and TNF- α in whites appears to be consistent with the result from a population-based study of 1,123 subjects in Italy. This study in Italy reported that plasma marine n-3 FA have inverse associations with proinflammatory cytokines, that is, IL-6 and TNF- α and positive associations with anti-inflammatory cytokines, that is, IL-10 (Ferrucci and others 2006). Thus, it may be possible that with a much larger sample we may observe significant associations of marine n-3 FA with IL-8, TNF- α , and other cytokines.

It is noted that serum levels of IP-10 in both the JJ and JA are higher than those in whites. Much higher prevalence of hypertension in the JJ as compared with whites may in part explain the higher levels of IP-10 in the JJ. A recent study reported that those with hypertension have higher levels of serum IP-10 as compared with those with normal BP (Antonelli and others 2008). In our study, serum levels of IP-10 were higher in those with hypertension than without in the JJ but not in whites: median (interquartile) levels for those with and without hypertension were 271 (229, 364) and 241 (191, 311), respectively, for the JJ ($P = 0.08$), 207 (150, 285), and 155 (116, 213), respectively, for the JA ($P < 0.01$), and 172 (116, 350) and 200 (153, 267), respectively, for whites (not

significant). Although serum levels of IP-10 are increased in various autoimmune diseases, for example, type 1 diabetes (Shimada and others 2001; Lee and others 2009), it is unlikely that higher serum levels of IP-10 are due to the difference in prevalence of autoimmune diseases because we excluded those with type 1 diabetes. Future study needs to determine levels of interferon- γ because IP-10 is secreted by several cell types in response to interferon- γ (Lee and others 2009).

Our observation of strong correlations between IL-4 and IL-8, between IL-4 and IL-1 α , and between IL-1 α and IL-8 is consistent with the results from a previous report in women (Wong and others 2008). The observed strong correlations are reasonable because these 3 cytokines are proinflammatory and contribute to similar inflammatory pathways.

We found that the JJ had significantly lower circulating levels of most proinflammatory cytokines, that is, IL-1 α , IL-4, IL-8, TNF- α , and MCP-1 than whites. The lower levels of the cytokines are unlikely due primarily to genetic factors because whites and JA had similar levels of these cytokines except for TNF- α and MCP-1. The lower levels of the cytokines are unlikely due to the difference in obesity between the JJ and whites because these cytokines had no significant correlation to obesity in either of the 2 samples. We could not deny the possibility that the lower levels of the cytokines in the JJ compared with whites are due to the difference in levels of marine n-3 FA because some of these cytokines had inverse associations with marine n-3 FA. With this small sample size, however, we were unable to test this hypothesis. Further investigation is needed.

We observed that the JJ had higher prevalence of hypertension and diabetes than whites in the United States even though the JJ are much less obese. These observations, however, do not contradict the results from previous reports. We have reported that prevalence of hypertension in Japan is one of the highest among developed countries (Sekikawa and Hayakawa 2004; Ueshima and others 2008). The higher prevalence of hypertension in Japan is partly because of higher dietary intake of salt in Japan (Stamler and others 2003). We have reported that prevalence of type 2 diabetes in Japan and the US are similar although the JJ are much less obese (Sekikawa and others 2000). This is because Asians including the Japanese are more susceptible to developing diabetes as migrant studies from Asia to the US showed (Fujimoto 1992).

Several limitations of the study warrant discussion. Generally we did not find significant association of circulating levels of cytokines with indices of obesity or serum marine n-3 FA in either sample. The weak and nonsignificant associations may be due to our relatively small sample size. It may be also due to a large within-individual variation in some cytokines, that is, IL-1ra, IP-10, MCP-1, and TNF- α as we have previously reported (Wong and others 2008). Additionally the weak nonsignificant associations, especially in IL-1 α , IL-4, and IL-8, may be due to the fact that levels of these cytokines in many subjects were under the detection levels. We cannot deny the possibility that the low levels of some cytokines in the JJ are due to the gene environmental interaction because difference in frequencies of promoter polymorphisms of some cytokines between the JJ and whites are reported, for example, TNF- α (Higuchi and others 1998; Skoog and others 1999; Grutters and others 2002)

and MCP-1 (Butcher and Picker 1996; Jibiki and others 2001). We examined only men aged 40–49 and our results are not generalizable to women or other age groups.

In conclusion, we found that in men aged 40–49 the JJ had circulating levels of many cytokines lower than those in whites and JA. Generally examined cytokines had weak and nonsignificant associations with indices of obesity and nonsignificant associations with marine n-3 FA. Further investigation is needed to determine why JJ had lower circulating levels of cytokines.

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Author Disclosure Statement

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