# Relationships Between Serum Unsaturated Fatty Acids and Coronary Risk Factors

## Negative Relations Between Nervonic Acid and Obesity-Related Risk Factors

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**SUMMARY**

Relative increases in unsaturated fatty acids (USFA) in the diet are considered to exert beneficial effects on coronary risk factors (CRF). However, detailed analysis of the relationships between serum USFA and CRF are scanty and there is no report of the relationship between nervonic acid (NA) and CRF. The objective of the present study was to analyze the relationships between serum USFA and CRF.

Body height and weight, blood pressure, fasting serum total cholesterol (TC), triacylglycerol (TG), HDL cholesterol (HDLc), fasting blood sugar (FBS), total fatty acid composition, leptin, and high-sensitivity C-reactive protein (CRP) were measured in 31 men (age, 41-78 years) and 11 women (age, 54-77 years). The relationships between serum USFA, and body mass index (BMI), leptin, systolic blood pressure (SBP), diastolic blood pressure (DBP), TC, TG, HDLc, FBS, and CRP were analyzed using multiple regression analysis. The final results were summarized using coronary risk factor scores (CRFS) in order to assess the correlations between USFA with CRF.

Oleic acid (OA), linoleic acid (LA), and eicosapentaenoic acid (EPA) were positively related to coronary risk factors (total CRFS = 2, 3, and 4, respectively), while nervonic acid (NA) exerted negative effects on these risk factors (total CRFS = −6 ). It is concluded NA may have preventive effects on obesity-related metabolic disorders. (Int Heart J 2005; 46: 975-985)

**Key words:** Leptin, Metabolic syndrome, Obesity, Diabetes mellitus

OBESITY, hypertension, diabetes mellitus (DM), dyslipidemia, and insulin resistance (metabolic syndrome) are increasing in frequency and becoming major causes of cardiovascular diseases.[1)] The excess intake of energy and a sedentary lifestyle are major causes of these morbid conditions. Excess saturated fatty acids

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(SF) and a relative deficiency in unsaturated fatty acids (USFA), especially n-3 polyunsaturated fatty acids (PUFA), have been identified as contributing factors.[2] However, detailed studies on the relationships between serum USFA and coronary risk factors (CRF) are limited. In this report, we analyzed the relationships between serum USFA, and body mass index (BMI),[3] leptin,[4] systolic blood pressure (SBP), diastolic blood pressure (DBP),[5] total cholesterol (TC), triacylglycerol (TG),[6] HDL cholesterol (HDLc), fasting blood sugar (FBS),[7] and high-sensitivity C-reactive protein (CRP)[8] (collectively defined as CRF in this report).

## METHODS

**Subjects:** Thirty-one males (age, 41-78 years) and 11 females (age, 54-77 years) without an existing or history of overt atherosclerotic cardiovascular disease, or overt diabetes mellitus who were enrolled in the control group in a previous case-control study on n-3 PUFA as a negative risk factor for myocardial infarction,[9] were the subjects in the present study. Written informed consent was obtained from all subjects and the ethics committee of all hospitals approved the study.

**Measurements:** Fasting blood samples were obtained from all subjects. Body height and weight, blood pressure, fasting blood sugar (FBS), total cholesterol (TC), triacylglycerol (TG), and HDL cholesterol (HDLc) were measured at each of the participating hospitals (see Appendix). Serum total fatty acid composition, CRP, and leptin were measured at a commercial laboratory center in Japan (SRL Inc.). Serum total fatty acid composition was measured using Omegawax 250 (SUPELCO, gas-chromatography), and serum leptin was measured using a Human Leptin RIA Kit (LINCO Research Inc.).

**Statistical analysis:** The mean and SD of each variable were calculated separately by gender, and sexual differences were examined with unpaired *t*-tests. *P* values less than 0.05 were considered to be significant. BMI, leptin, SBP, DBP, TC, TG, HDLc, FBS, and CRP were analyzed by multiple regression analysis using the combination of oleic acid (OA, C18:1:n-9), linoleic acid (LA, C18:2:n-6), linolenic acid (LNA, C18:3:n-3), arachidonic acid (AA, C20:4:n-6), eicosapentaenoic acid (EPA, C20:5:n-3), and nervonic acid (NA, C24:1:n-9), or the combination of total n-9 monounsaturated fatty acids (MUFA) (Σn9), total n-6 polyunsaturated fatty acids (PUFA) (Σn6), and total n-3 PUFA (Σn3) as independent variables. Other USFA and total saturated fatty acids (ΣSF) were omitted from the independent variables in order to avoid multicolinearity. Both fatty acid data expressed in $\mu$g/mL and wt% (weight % of individual fatty acid/total fatty acids) were analyzed separately. The former values express the absolute concentrations of fatty acids, while the latter express their relative quantities. *P* values less than 0.05 were considered to be significant. The coronary risk factor score

(CRFS) was defined as follows. Independent variables were given a score, plus one, with each risk factor except for HDLc, when the coefficients were significantly positive, and given a score, minus one, when the coefficients were significantly negative. For HDLc, the score was minus one when the coefficients were significantly positive and plus one when the coefficients were significantly negative. The CRFS for each individual variable was defined as the summation of the scores.

## RESULTS

**Baseline data:** The mean and SD of the coronary risk factors are presented in Table I. There were significant gender differences in leptin and HDLc. The mean and SD of each fatty acid and each fatty acid class are presented in Table II. There were no significant gender differences in any variable.

**Multiple regression of CRF using USFA and fatty acid class ($\mu$g/mL) separately as independent variables:** The results of multiple regression analysis of CRF using USFA and fatty acid class in $\mu$g/mL separately as independent variables are shown in Table III. OA was significantly positively correlated to TG and significantly negatively correlated to HDLc. LA was significantly positively correlated to leptin and TC, and EPA was significantly positively correlated to BMI, leptin, TC, and FBS. NA was significantly negatively correlated to leptin. With respect to fatty acid class, Σn9 was significantly positively correlated to TG and significantly negatively correlated to TC and HDLc, while Σn6 was significantly negatively correlated to TG and significantly positively correlated to leptin, TC, and HDLc. Σn3 was significantly positively correlated to SBP and TC.

**Table I.** Basal Data of Multiple Coronary Risk Factors

| | Male[1] | | Female[2] | | |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | *P*[3] |
| Age (years) | 57.4 | 10.7 | 61.4 | 7.1 | NS |
| BMI (kg/m$^2$) | 24.8 | 3.8 | 23.6 | 3.7 | NS |
| leptin (ng/mL) | 5.3 | 2.5 | 7.6 | 4.7 | < 0.05 |
| SBP (mmHg) | 127 | 20 | 131 | 25 | NS |
| DBP (mmHg) | 73 | 13 | 76 | 17 | NS |
| TG (mg/dL) | 122 | 76 | 118 | 43 | NS |
| TC (mg/dL) | 202 | 29 | 223 | 40 | NS |
| HDLc (mg/dL) | 57 | 14 | 70 | 17 | < 0.05 |
| FBS (mg/dL) | 101 | 13 | 97 | 17 | NS |
| CRP (ng/mL) | 2265 | 6836 | 1107 | 1560 | NS |

[1]*n* = 32, [2]*n* = 11, [3]Calculated with unpaired *t*-tests. Values more than 0.05 are presented as NS (not significant).

**Table II.** Basal Data of Fatty Acid Composition

| | Male[1] | | Female[2] | | | | Male[1] | | Female[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $\mu$g/mL | mean | SD | mean | SD | $P$[3] | wt% | mean | SD | mean | SD | $P$[3] |
| C12:0 | 1.4677 | 1.336 | 2.282 | 1.99 | NS | C12:0 | 0.042 | 0.029 | 0.062 | 0.046 | NS |
| C14:0 | 28.497 | 14.59 | 35.03 | 17.4 | NS | C14:0 | 0.832 | 0.247 | 0.945 | 0.354 | NS |
| C16:0 | 770.65 | 244.6 | 847.2 | 212.7 | NS | C16:0 | 23.2 | 1.61 | 23.24 | 2.238 | NS |
| C16:1:n7 | 69.294 | 37.2 | 80.41 | 35.57 | NS | C16:1:n7 | 2.024 | 0.651 | 2.188 | 0.745 | NS |
| C18:0 | 233.43 | 53.3 | 264.4 | 50.55 | NS | C18:0 | 7.165 | 0.54 | 7.32 | 0.462 | NS |
| C18:1:n9 | 658.45 | 263.9 | 668 | 139 | NS | C18:1:n9 | 19.47 | 2.574 | 18.49 | 1.847 | NS |
| C18:2:n6 | 896.08 | 225.1 | 988.3 | 206.7 | NS | C18:2:n6 | 27.5 | 3.739 | 27.44 | 3.944 | NS |
| C18:3:n6 | 10.7 | 7.253 | 13.2 | 5.647 | NS | C18:3:n6 | 0.311 | 0.161 | 0.356 | 0.114 | NS |
| C18:3:n3 | 29.016 | 15.03 | 32.74 | 12.41 | NS | C18:3:n3 | 0.863 | 0.242 | 0.887 | 0.221 | NS |
| C20:0 | 8.0065 | 1.883 | 8.391 | 1.466 | NS | C20:0 | 0.248 | 0.044 | 0.235 | 0.038 | NS |
| C20:1:n9 | 6.0129 | 2.535 | 6.036 | 1.556 | NS | C20:1:n9 | 0.181 | 0.044 | 0.167 | 0.029 | NS |
| C20:2:n6 | 6.3194 | 1.708 | 6.718 | 2.256 | NS | C20:2:n6 | 0.194 | 0.025 | 0.181 | 0.035 | NS |
| C20:3:n6 | 33.048 | 13.5 | 36.49 | 14.11 | NS | C20:3:n6 | 0.997 | 0.282 | 0.991 | 0.281 | NS |
| C20:4:n6 | 161.93 | 46.18 | 191.4 | 41.69 | NS | C20:4:n6 | 5.005 | 1.042 | 5.356 | 0.971 | NS |
| C20:5:n3 | 109.23 | 63.61 | 127.1 | 55.8 | NS | C20:5:n3 | 3.417 | 1.92 | 3.477 | 1.367 | NS |
| C22:0 | 19.265 | 5.106 | 21.03 | 4.484 | NS | C22:0 | 0.595 | 0.123 | 0.586 | 0.097 | NS |
| C22:1:n9 | 2.4839 | 1.139 | 2.182 | 0.84 | NS | C22:1:n9 | 0.08 | 0.042 | 0.061 | 0.028 | NS |
| C22:4:n6 | 3.6935 | 1.397 | 4.1 | 1.081 | NS | C22:4:n6 | 0.112 | 0.026 | 0.114 | 0.022 | NS |
| C22:5:n3 | 26.194 | 10.37 | 27.7 | 8.322 | NS | C22:5:n3 | 0.794 | 0.221 | 0.761 | 0.134 | NS |
| C22:6:n3 | 17.49 | 4.012 | 17.77 | 3.329 | NS | C22:6:n3 | 0.546 | 0.103 | 0.497 | 0.073 | NS |
| C24:0 | 165.33 | 54.36 | 195.4 | 46.63 | NS | C24:0 | 5.129 | 1.507 | 5.417 | 0.799 | NS |
| C24:1:n9 | 38.787 | 7.044 | 41.1 | 5.068 | NS | C24:1:n9 | 1.24 | 0.316 | 1.175 | 0.253 | NS |
| ΣSF[4] | 1078.8 | 314.6 | 1196 | 279.6 | NS | ΣSF[4] | 32.63 | 1.477 | 32.89 | 2.52 | NS |
| Σn9[5] | 707.61 | 268.1 | 719.2 | 141.1 | NS | Σn9[5] | 20.71 | 2.382 | 19.67 | 1.869 | NS |
| Σn6[6] | 1111.8 | 271.3 | 1240 | 242.1 | NS | Σn6[6] | 34.12 | 4.214 | 34.43 | 4.09 | NS |
| Σn3[7] | 329.76 | 131 | 382.8 | 108.4 | NS | Σn3[7] | 10.2 | 3.596 | 10.54 | 2.02 | NS |

[1]$n$ = 31, [2]$n$ = 11

[3]Calculated with unpaired $t$-tests. Values more than 0.05 are presented as NS (not significant).

[4]total saturated fatty acids, [5]total n-9 monounsaturated fatty acids,

[6]total n-6 polyunsaturated fatty acids, [7]total n-3 polyunsaturated fatty acids.

**Multiple regression analysis of CRF using USFA and fatty acid class (wt%) separately as independent variables:** The results of multiple regression analysis of CRF using USFA and fatty acid class (wt%) as independent variables are shown in Table IV. OA was significantly negatively correlated to leptin and significantly positively correlated to TG. LA was significantly positively correlated to TC, and NA was significantly negatively correlated to BMI, leptin, TG, TC, and FBS. Significant negative correlations were found between leptin and all three unsaturated fatty acid classes, and between HDLc and Σn9. No significant correlation was found between LNA, AA, EPA, and CRF.

**Total CRFS:** Among individual USFA, OA, LA, and EPA had positive total CRFS (2, 3, and 4, respectively), while NA had a negative total CRFS (–6). As

**Table III.** Multiple Regressions for Risk Factors Using USFA (unsaturated fatty acids) or Fatty Acid Classes ($\mu$g/mL) as Independent Variables[1]

| $\mu$g/mL | BMI | | Leptin | | SBP | | DBP | | TG | | TC | | HDLc | | FBS | | CRP | | CRFS[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | |
| intercept | 23.71 | $< 10^{-6}$ | 4.6652 | NS | 99.66 | $< 10^{-4}$ | 45.15 | <0.01 | 40.96 | NS | 68.14 | < 0.01 | 45.42 | < 0.01 | 81.51 | $< 10^{-7}$ | 4476 | NS | |
| OA[5] | 0.005 | NS | -0.001 | NS | 0.002 | NS | 0.011 | NS | 0.273 | $< 10^{-8}$ | -0.01 | NS | -0.04 | < 0.05 | 0.028 | NS | -2.66 | NS | 2 |
| LA[6] | 0.008 | NS | 0.0098 | < 0.05 | 0.023 | NS | 0.026 | NS | -0.07 | NS | 0.143 | $< 10^{-5}$ | 0.034 | NS | 0.027 | NS | -4.52 | NS | 2 |
| LNA[7] | -0.08 | NS | -0.038 | NS | -0.37 | NS | -0.12 | NS | 0.94 | NS | -0.78 | NS | -0.23 | NS | -0.11 | NS | -31.1 | NS | 0 |
| AA[8] | -0.02 | NS | 0.0065 | NS | -0.04 | NS | -0.08 | NS | -0.13 | NS | 0.027 | NS | 0.135 | NS | -0.08 | NS | 10.95 | NS | 0 |
| EPA[9] | 0.024 | < 0.05 | 0.0189 | < 0.05 | 0.117 | NS | 0.008 | NS | 0.106 | NS | 0.177 | < 0.01 | 0.07 | NS | 0.074 | < 0.05 | 5.736 | NS | 4 |
| NA[10] | -0.16 | NS | -0.234 | < 0.01 | 0.281 | NS | 0.319 | NS | -1.44 | NS | 0.398 | NS | -0.31 | NS | -0.39 | NS | 46.37 | NS | -1 |
| intercept | 19.53 | $< 10^{-7}$ | -1.578 | NS | 106 | $< 10^{-7}$ | 51.15 | $< 10^{-4}$ | -19.7 | NS | 81.86 | $< 10^{-5}$ | 41.77 | $< 10^{-3}$ | 68.65 | $< 10^{-8}$ | 6153 | NS | |
| $\Sigma$n9[11] | 0.002 | NS | -0.002 | NS | -0.02 | NS | 0.002 | NS | 0.31 | $< 10^{-9}$ | -0.06 | < 0.01 | -0.06 | < 0.01 | 0.023 | NS | -4.89 | NS | 1 |
| $\Sigma$n6[12] | 0.001 | NS | 0.006 | < 0.05 | 0.016 | NS | 0.017 | NS | -0.09 | < 0.05 | 0.128 | $< 10^{-6}$ | 0.041 | < 0.01 | 0.004 | NS | -1.46 | NS | 0 |
| $\Sigma$n3[13] | 0.006 | NS | 0.0065 | NS | 0.058 | < 0.05 | 0.006 | NS | 0.067 | NS | 0.072 | < 0.05 | 0.032 | NS | 0.028 | NS | 2.777 | NS | 2 |

[1]$n$ = 42. [2]Independent variables were given a score, plus one, with each risk factor except for HDLc when the coefficient was significantly positive, and given a score, minus one, when the coefficient was significantly negative. As for HDLc the score was minus one when the coefficient was significantly positive and plus one when the coefficient was significantly negative. CRFS for each individual variable was defined as the summation of the scores. [3]partial regression coefficient. [4]Values more than 0.05 are presented as NS (not significant). [5]oleic acid, [6]linoleic acid, [7]linolenic acid, [8]arachidonic acid, [9]eicosapentaenoic acid, [10]nervonic acid, [11]total n-9 monounsaturated fatty acids, [12]total n-6 polyunsaturated fatty acids, [13]total n-3 polyunsaturated fatty acids.

**Table IV.** Multiple Regressions for Risk Factors Using USFA or Fatty Acid Classes (wt%) as Independent Variables[1]

| wt% | BMI | | Leptin | | SBP | | DBP | | TG | | TC | | HDLc | | FBS | | CRP | | CRFS[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | $\beta$[3] | $P$[4] | |
| intercept | 39.62 | < 0.05 | 39.683 | < 0.01 | 240.7 | < 0.05 | 94.58 | < 0.05 | 33.42 | NS | 55.11 | NS | 131.17 | NS | 29.42 | NS | 39789 | NS | |
| OA[5] | -0.12 | NS | -0.79 | < 0.05 | -2.36 | NS | 0.237 | NS | 11.42 | < 0.05 | 3.469 | NS | -3.32 | NS | 2.675 | NS | -1102 | NS | 0 |
| LA[6] | 0.059 | NS | -0.148 | NS | -0.9 | NS | 0.528 | NS | -2.07 | NS | 5.525 | < 0.05 | 0.0468 | NS | 1.487 | NS | -555 | NS | 1 |
| LNA[7] | -2.59 | NS | -0.206 | NS | -16.5 | NS | -7.88 | NS | 61.13 | NS | -21.6 | NS | -9.151 | NS | 2.877 | NS | -4221 | NS | 0 |
| AA[8] | -1.21 | NS | -0.583 | NS | -3.65 | NS | -3.79 | NS | -4.58 | NS | 0.209 | NS | 3.1171 | NS | -2.17 | NS | 110.5 | NS | 0 |
| EPA[9] | 0.302 | NS | -0.563 | NS | 0.69 | NS | -0.15 | NS | 3.846 | NS | 8.998 | NS | 0.1979 | NS | 3.959 | NS | -484 | NS | 0 |
| NA[10] | -5.73 | < 0.05 | -7.723 | $< 10^{-4}$ | -9.74 | NS | -10.6 | NS | -96.6 | $< 10^{-3}$ | -64.6 | < 0.01 | -14.09 | NS | -22.2 | < 0.01 | 2752 | NS | -5 |
| intercept | 55.05 | < 0.05 | 56.886 | < 0.01 | 221.5 | NS | 106.4 | NS | 199.2 | NS | 103.9 | NS | 153.93 | NS | 32.66 | NS | 38795 | NS | |
| $\Sigma$n9[11] | -0.44 | NS | -0.992 | < 0.05 | -2.28 | NS | -0.05 | NS | 10.75 | NS | 1.81 | NS | -3.848 | < 0.05 | 2.592 | NS | -1167 | NS | 0 |
| $\Sigma$n6[12] | -0.5 | NS | -0.642 | < 0.01 | -1.35 | NS | -0.62 | NS | -7.76 | NS | 1.762 | NS | -0.221 | NS | 0.083 | NS | -315 | NS | -1 |
| $\Sigma$n3[13] | -0.44 | NS | -0.847 | < 0.01 | -0.08 | NS | -0.99 | NS | -3.16 | NS | 0.643 | NS | -0.745 | NS | 1.081 | NS | -215 | NS | -1 |

[1]$n$ = 42. [2]Independent variables were given a score, plus one, with each risk factor except for HDLc when the coefficient was significantly positive, and given a score, minus one, when the coefficient was significantly negative. As for HDLc the score was minus one when the coefficient was significantly positive and plus one when the coefficient was significantly negative. CRFS for each individual variable was defined as the summation of the scores. [3]partial regression coefficient. [4]Values more than 0.05 are presented as NS (not significant). [5]oleic acid, [6]linoleic acid, [7]linolenic acid, [8]arachidonic acid, [9]eicosapentaenoic acid, [10]nervonic acid, [11]total n-9 monounsaturated fatty acids, [12]total n-6 polyunsaturated fatty acids, [13]total n-3 polyunsaturated fatty acids.

for fatty acid classes, n-6 PUFA had a negative total CRFS (–1), while n-9 MUFA and n3 PUFA had positive total scores.[1)]

## DISCUSSION

The relationships between USFA and coronary risk factors are controversial. Kris-Etherton, *et al* concluded that, although not all epidemiologic studies have demonstrated a beneficial association between PUFA and cardiovascular disease, n-6 PUFA have the most potent cholesterol-lowering effects of the individual fatty acid classes in their review.[10)] Bemelmans, *et al* reported in their longitudinal study that increased LNA intakes resulted in lower HDLc and higher TG than did increased LA intakes.[11)] Djousse, *et al* reported in their cross-sectional study that consumption of total LNA was inversely related to TG.[12)] Zhao, *et al* reported in their interventional study that both an LA diet and LNA diet decreased TC, LDL cholesterol, and TG similarly and that an LNA diet decreased CRP and HDLc.[13)] However, these dietary studies are different from studies investigating the associations between serum fatty acid composition with CRF defined above, though there are some correlations between diet and plasma fatty acid composition. Ma, *et al* reported in their cross-sectional dietary intake study[14)] that the Pearson correlations between dietary and plasma fatty acid (expressed as % of total fatty acids) for phospholipid (PL) and cholesterol ester (CE), respectively, were as follows: saturated fatty acids (r = 0.15 and 0.23), MUFA (r = 0.05 and 0.01), PUFA (r = 0.25 and 0.31), LA (r = 0.22 and 0.28), LNA (r = 0.15 and 0.21), EPA (r = 0.20 and 0.23), and DHA (r = 0.42 and 0.42), (*n* = 3570). Wang, *et al* prospectively investigated the correlations between plasma CE and PL fatty acid composition with the incidence of coronary heart disease (CHD) and reported that in both CE and PL fractions, the proportions (% of total fatty acid) of stearic acid, D γLNA, and ΣSF were significantly higher while AA and total PUFA were significantly lower among patients with CHD.[15)] LNA in the CE fraction was also significantly positively correlated to CHD and monounsaturated fatty acids were not associated with CHD in their report.[15)]

In the present cross-sectional study, among individual USFA, OA, LA, and EPA were positively correlated to CRF (CRFS = 2, 2, and 4, respectively), while NA was negatively correlated to CRF (CRFS = –1), when expressed as absolute concentrations ($\mu$g/mL). As for the class of fatty acid, n-6 PUFA had a negative CRFS (= –1), while both n-3 PUFA and n-9 MUFA had a positive CRFS (= 1) (Table III). When expressed as proportions of total fatty acids (wt%), LA was positively correlated to CRF (CRFS = 1), while NA was negatively correlated to CRF (CRFS = –5). With respect to fatty acid class, n-9 MUFA had a neutral CRFS (= 0), while both n-6 PUFA and n-3 PUFA had a negative CRFS (= –1)

(Table IV). Therefore, OA, LA, and EPA had overall positive correlations to CRF (total CRFS = 2, 3, and 4, respectively), while NA had an overall negative correlation to CRF (total CRFS = −6). With respect to fatty acid class, n-6 PUFA had a negative total CRFS (= −1), while both n-3 PUFA and n-9 MUFA had a positive total CRFS (= 1). Relative concentrations (wt%) of USFA may mainly reflect their favorable nature as regulators of metabolism such as ligands for peroxisome proliferator-activated receptors,[16,17] while their absolute concentrations may mainly reflect their property as fuel rather than information molecules. We must consider both these characteristics in dietary or supplementary strategies. Therefore, we propose total CRFS as a measure for an overall assessment. The overall favorable correlation between n-6 PUFA and CRF may explain the results of Wang, *et al*, who found AA was inversely related to CHD.[15] Though having a positive CRFS in our study, n-3 PUFA has been shown to reduce the incidence of cardiovascular diseases and sudden death, and consequently has been recommended in the American Heart Association Dietary Guidelines.[18] Therefore, the beneficial effects of n-3 PUFA or EPA on CHD may be related to factors other than CRF such as ion channels,[19] plaque stability,[20] myocardial oxygen consumption,[21] eicosanoids,[22] platelet aggregation,[23] endothelial function,[24] and Src family kinase inhibition.[25] From the standpoint of dietary evolution and eicosanoid metabolism, Simopoulos proposed adopting the n-6/n-3 PUFA ratio of 1-2/1, while current estimates in Western cultures suggest an n-6/n-3 PUFA ratio of 10-20/1.[22] On the other hand, Wijendran, *et al* recommended an n-6/n-3 ratio of 6/1 as an achievable level for most healthy adults in Western cultures.[26] However, Brady, *et al* reported in their interventional study that the background dietary n-6 PUFA concentration did not modulate the effect of n-3 PUFA supplementation on blood lipids or measures of insulin sensitivity,[27] and Mozaffarian, *et al* reported in their longitudinal study that n-3 PUFA may reduce coronary heart disease risk, with little apparent influence from background n-6 PUFA intake.[28] These observations may reflect the beneficial relation of n-6 PUFA with CRF detected in our study. Although n-3 PUFA is established as a protective nutritional substance against sudden death,[29,30] relationships between n-3 PUFA and nonfatal myocardial infarction are controversial.[31-33] Guallar, *et al* reported that there was no difference in plasma fish oil levels between patients with myocardial infarction and the control in a prospective nested case-control study (the Physician's Health Study),[31] while Tavani, *et al* showed in their case-control study that n-3 PUFA was inversely related to nonfatal myocardial infarction.[33] We reported that n-3 PUFA may be negatively related to nonfatal myocardial infarction and the optimal n-6/n-3 PUFA ratio may be lower than 3/1 in a case-control study[9] conducted in Japan where the incidence of myocardial infarction is low and the mean ratio of n-6/n-3 PUFA is about 3/1. Siscovick, *et al* suggested

a possible threshold for n-3 PUFA intake (the equivalent of one fatty fish meal a week) for a marked reduction in cardiac sudden death.[34] We suggest another higher possible threshold for a reduction in nonfatal myocardial infarction, because the protective effects of n-3 PUFA from nonfatal myocardial infarction are obscure in Western cultures where n-3 PUFA intake is very low,[29,31,32] whereas these effects may be evident in epidemiological studies comparing different (in regard to fish consumption) ethnic groups[35] and the protective effect was observed at very high levels of n-3 PUFA compared to those in Western cultures in our previous study.[9]

NA, as well as DHA, is well-known as a major component of brain phospholipids, and has been suggested as a therapeutic supplement for demyelinating diseases by some investigators.[36] However, it is catabolized in peroxisomes as are other very long chain fatty acids, and this process is defective in some demylinating diseases, so, the efficacy of dietary NA therapy has been questioned by others.[37] There are no previous reports concerning the association between NA with CRF or CHD. Our study may be the first report describing negative associations for NA with BMI, leptin, TG, TC, and FBS. Bang, *et al*[35] pointed out the following two facts in their report. "The polyunsaturated fatty acids were predominantly of the linolenic class (n-3) in Eskimo diets and the linoleic class (n-6) in Danes. Monoenes other than palmitoleic and oleic acids were high in Eskimo diets, but negligible in Danish". Most of the monoenes other than OA and POA in Eskimo diets described by Bang, *et al* were C20:1 and C22:1, which shortens to C18:1:n-9 (OA) or elongates to C24:1:n-9 (NA) *in vivo*.[38] According to the report by Dyerberg, *et al*[39] the average plasma fatty acid compositions of MUFA in Greenland Eskimos were OA = 25% > POA(C16:1:n-9) = 9% > NA = 0.88% > C20:1 = 0.05% > other MUFA in cholesterol esters, OA = 35% > POA = 9.47% > C20:1 = 3.35% > NA = 0.15% > C14:1 = 0.04% > C22:1 = 0.01% > other MUFA in triacylglycerols including free fatty acids and OA = 16% > C20:1 = 2.73% > POA = 2.68% > NA = 0.13% > other MUFA in phospholipids. In our data, the average serum total fatty acid composition of MUFA was OA = 20% > POA = 2.2% > NA = 1.3% > EA (C20:1:n-9) = 0.2% > ERA (C22:1:n-9) = 0.07% > other MUFA. In certain species C22:1 depresses body weight gain mainly through an anorectic effect and causes cardiac fibrosis due to lipid accumulation.[40] However, it is known that adaptation occurs to low doses[35] or to prolonged exposure to C22:1,[41] Though possibly toxic in large doses,[40] certain doses of certain very long chain MUFA, including NA, may not only be nontoxic in humans,[42] but also lead to prevention from obesity-related metabolic disorders, as the results of the present study suggest, and, together with n-3 PUFA, provide protection against coronary heart disease, as is the case in Eskimo diets.[35] Dietary intervention studies on the relationship between NA and obesity-related meta-

bolic disorders may be warranted.

**Limitations:** The size of the present study population was rather small and the measurement procedures for some variables were not precisely defined. Therefore, the values could include undue errors. However, these errors might lead to nonsignificant results rather than significant associations. Therefore, the significant results in our present study may be relevant.

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## APPENDLX

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