BJN British Journal of Nutrition

pressure, cholesterol (total, HDL, and LDL), fasting TAG, fasting glucose, and glycated Hb. All statistical analyses were performed using SAS statistical software (version 8.2; SAS Institute Inc., Cary, NC, USA). Linear regression models were constructed using the PROC GLM procedure to examine the association between dietary acid–base load measures with cardiometabolic risk factors. For the analyses, subjects were categorized into quintiles according to the dietary acid–base load measures. The mean metabolic risk factor values (with standard errors) were calculated by quintiles of dietary acid–base load measures after multivariate adjustment for potential confounding factors. Confounding factors included residential block, size of residential area, survey year (2006 or 2007; because of the different laboratories with different kits used for blood analyses for the 2006 and 2007 surveys, even though there were no differences in the assay methods), current smoking, and physical activity (continuous). BMI (continuous) was added as a confounding factor in all analyses except for that for BMI itself. Waist circumference (continuous) was also added as a confounding factor in the analyses except for those for BMI and waist circumference. We initially intended to include estimated excretion of OA (the diet-independent acid–base load) as a confounding factor to investigate diet-dependent acid–base load (PRAL and Pro:K) and cardiometabolic risk factors. However, OA was strongly correlated with body height and weight and hence BMI (Pearson correlation coefficient with OA = 0·70, 0·95, and 0·68, respectively), as OA is just a function of the combination of height and weight as mentioned earlier. To avoid over-adjustment, we consequently did not include OA as a confounding factor. Since alcohol intake was extremely low (mean = 1·5 g/d), it was not considered as a confounding factor. Because the inclusion of measures of obesity (BMI, waist circumference, or both) as confounding factors did not influence the results materially, we present the full-adjustment models only. Linear trends with increasing levels of dietary acid–base load measures were tested for by assigning each participant a median value for the category and modelling this value as a continuous variable. All reported P values are two-tailed, and a value of $P<0·05$ was considered statistically significant.

Results

Basic characteristics of all subjects (n 1136; those included in the analyses of BMI, waist circumference, and systolic and diastolic blood pressure) are shown in Table 1. Mean PRAL was 10·4 mEq/d, and mean Pro:K was 1·23 g/mEq. There was a strong correlation between these variables (Pearson correlation coefficient = 0·84). The potential confounding variables for all subjects are shown in Table 2 according to quintile of dietary acid–base load measure. Both PRAL and Pro:K were associated negatively with physical activity and positively with waist circumference. There was also a positive association between Pro:K and BMI. The dietary intakes of all subjects are shown in Table 3 according to quintile of dietary acid–base load measures. PRAL was associated positively with protein and negatively with K, Ca and Mg as well as P, while Pro:K was positively associated with K as well as protein. For foods, both PRAL and Pro:K showed a positive association with meats, eggs, cereals, and fish and shellfish, and a negative association with fruits, vegetables and dairy products. According to the quintiles of the dietary acid–base load measures, similar patterns were observed for potential confounding factors and dietary intake among those subjects included in the analyses of cholesterol (total, HDL, and LDL) and glycated Hb (n 1121), fasting TAG (n 1088), and fasting glucose (n 1089) (data not shown).

The multivariate-adjusted mean values for cardiometabolic risk factors across quintiles of dietary acid–base load

Table 1. Basic characteristics of subjects (n 1136)*
(Mean values and standard deviations)

	Mean	SD
Age (years)	19·6	1·1
Body height (cm)	158·4	5·5
Body weight (kg)	53·6	7·7
BMI (kg/m^2)	21·3	2·7
Waist circumference (cm)	72·9	7·1
Systolic blood pressure (mmHg)	106·4	10·6
Diastolic blood pressure (mmHg)	69·3	8·2
Total cholesterol (mg/l)	1889	318
HDL-cholesterol (mg/l)	706	127
LDL-cholesterol (mg/l)	1070	272
Fasting TAG (mg/l)	611	288
Fasting glucose (mg/l)	840	64
Glycated Hb (%)	4·87	0·26
Residential block (%)		
North (Kanto, Hokkaido, and Tohoku)	56	
Central (Tokai, Hokuriku, and Kinki)	24	
South (Kyushu and Chugoku)	20	
Size of residential area (%)		
City with population ≥ 1 million	16	
City with population < 1 million	78	
Town and village	6	
Survey year (%)		
2006	41	
2007	59	
Current smoking (%)		
No	97	
Yes	3	
Physical activity (total MET-h/d)	33·9	3·1
Estimated urinary excretion of organic acid (mEq/d)	36·3	2·7
Energy intake (kJ/d)	7376	1874
Nutrient intake†		
Protein (g/d)	59·7	8·8
P (mg/d)	915	169
K (mg/d)	1971	471
Ca (mg/d)	502	171
Mg (mg/d)	213	49
Food intake (g/d)		
Meats	60·1	31·7
Fish and shellfish	50·3	28·6
Eggs	35·6	21·4
Dairy products	145·6	127·2
Fruits	57·9	57·3
Vegetables	206·9	121·2
Cereals	380·3	92·1
Measures of dietary acid–base load‡		
Potential renal acid load (mEq/d)	10·4	7·6
Pro:K (g/mEq)	1·23	0·22

MET, metabolic equivalents; Pro:K, ratio of dietary protein to K.
* n 1121 for cholesterol (total, HDL, and LDL) and glycated Hb; 1088 for fasting TAG; 1089 for fasting glucose.
† Energy-adjusted using the residual method.
‡ Calculated using crude nutrient intake values and then energy-adjusted using the residual method.

Table 2. Selected characteristics according to quintile of measures of dietary acid–base load (n 1136)
(Mean values and standard deviations)

	Quintile of measures of dietary acid–base load										
	1 (n 227)		2 (n 227)		3 (n 228)		4 (n 227)		5 (n 227)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P*
Potential renal acid load (mEq/d)†	−0·8	6·5	7·8	1·2	11·1	0·8	14·2	0·9	19·5	3·4	
Residential block (%)											0·69
North (Kanto, Hokkaido, and Tohoku)	57		55		54		57		58		
Central (Tokai, Hokuriku, and Kinki)	24		27		24		22		25		
South (Kyushu and Chugoku)	19		19		23		21		17		
Size of residential area (%)											0·036
City with population ≥ 1 million	16		15		13		15		22		
City with population < 1 million	76		80		79		79		74		
Town and village	7		5		9		6		3		
Survey year (%)											0·86
2006	44		36		42		41		41		
2007	56		64		58		59		59		
Current smoking (%)											0·11
No	96		98		97		98		98		
Yes	4		2		3		2		2		
Physical activity (total mEq-h/d)	34·4	3·5	33·8	2·5	33·8	3·4	33·6	2·4	33·8	3·3	0·021
BMI (kg/m^2)	21·1	2·6	21·2	3·2	21·5	2·5	21·6	2·6	21·3	2·7	0·11
Waist circumference (cm)	72·3	7·0	72·0	7·6	73·2	6·6	73·9	6·8	73·2	7·1	0·019
Pro:K (g/mEq)†	0·94	0·09	1·11	0·03	1·22	0·03	1·33	0·04	1·54	0·13	
Residential block (%)											0·14
North (Kanto, Hokkaido, and Tohoku)	56		57		60		55		51		
Central (Tokai, Hokuriku, and Kinki)	24		26		22		22		27		
South (Kyushu and Chugoku)	19		17		18		23		22		
Size of residential area (%)											0·084
City with population ≥ 1 million	15		14		16		18		18		
City with population < 1 million	78		78		77		78		78		
Town and village	7		8		7		5		4		
Survey year (%)											0·31
2006	44		38		41		41		38		
2007	56		62		59		59		62		
Current smoking (%)											0·59
No	96		98		98		97		97		
Yes	4		2		2		3		3		
Physical activity (total MET-h/d)	34·4	3·6	34·0	2·6	33·7	2·6	34·1	3·9	33·4	2·2	0·002
BMI (kg/m^2)	21·0	2·5	21·2	3·1	21·4	2·5	21·6	2·7	21·5	2·8	0·030
Waist circumference (cm)	72·1	7·0	72·2	7·4	72·8	6·3	73·8	7·0	73·6	7·4	0·002

MET, metabolic equivalents; Pro:K, ratio of dietary protein to K.
* For categorical variables, a Mantel–Haenszel χ^2 test was used; for continuous variables, a linear trend test was used with the median value in each quintile as a continuous variable in linear regression.
† Calculated using crude nutrient intake values and then energy-adjusted using the residual method.

measures are shown in Table 4. After adjustment for potential confounding factors, higher PRAL and Pro:K (more acidic dietary acid–base loads) were associated with higher systolic and diastolic blood pressure (mean difference between the lowest and highest quintiles = 2·1 mmHg (P for trend = 0·028) and 1·6 mmHg (P for trend = 0·035) for PRAL, and 2·5 mmHg (P for trend = 0·012) and 2·3 mmHg (P for trend = 0·009) for Pro:K, respectively). In addition, PRAL showed an independent positive association with total and LDL cholesterol (mean difference = 59 mg/l (P for trend = 0·042) and 60 mg/l (P for trend = 0·021), respectively). Pro:K was positively associated with BMI and waist circumference independently of potential confounding factors (mean difference = 0·5 kg/m^2 (P for trend = 0·024) and 0·8 cm (P for trend = 0·012), respectively). No significant associations were observed between PRAL or Pro:K and any of the other cardiometabolic risk factors examined.

Discussion

In a group of free-living young Japanese women, we found that higher PRAL and Pro:K (more acidic dietary acid–base loads) were associated with higher systolic and diastolic blood pressure after adjustment for possible confounding factors. We also found independent positive associations between PRAL and total and LDL-cholesterol, as well as between Pro:K and BMI and waist circumference. To our knowledge, this is the first study to examine the relationships between dietary acid–base load measures and cardiometabolic risk factors.

Consistent with previous studies[19–21], the correlation between PRAL and Pro:K was quite high (Pearson correlation coefficient = 0·84–0·93), which indicates that these measures capture similar, but not the same, elements of dietary acid–base load. Given that only blood pressure was

Table 3. Nutrient and food intake according to quintile of measures of dietary acid–base load (*n* 1136)
(Mean values and standard deviations)

	Quintile of measures of dietary acid–base load										
	1 (*n* 227)		2 (*n* 227)		3 (*n* 228)		4 (*n* 227)		5 (*n* 227)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P* for trend*
Potential renal acid load (mEq/d)†	−0·8	6·5	7·8	1·2	11·1	0·8	14·2	0·9	19·5	3·4	
Nutrient intake‡											
Protein (g/d)	58·5	10·8	59·0	8·4	58·8	7·2	59·4	7·3	62·9	9·1	<0·0001
P (mg/d)	942	203	922	154	896	154	890	150	924	173	0·043
K (mg/d)	2446	528	2067	361	1895	315	1771	318	1678	368	<0·0001
Ca (mg/d)	577	202	530	149	489	161	470	151	442	154	<0·0001
Mg (mg/d)	249	58	220	39	207	37	199	40	193	47	<0·0001
Food intake (g/d)‡											
Meats	49·8	28·7	55·6	24·6	59·7	27·6	61·3	29·4	73·8	41·1	<0·0001
Fish and shellfish	47·9	31·5	49·9	26·8	48·4	22·7	49·0	24·1	56·2	35·3	0·008
Eggs	31·5	21·9	32·3	18·1	34·5	19·3	37·3	20·1	42·7	25·0	<0·0001
Dairy products	156·4	142·9	167·1	132·0	142·3	128·8	138·6	118·7	123·3	107·5	0·001
Fruits	91·7	89·2	62·4	41·8	54·4	45·4	45·8	36·7	35·2	38·9	<0·0001
Vegetables	306·2	176·2	218·6	95·9	190·4	81·3	169·8	77·2	149·6	77·4	<0·0001
Cereals	344·8	94·5	376·0	79·9	380·9	83·6	394·4	89·4	405·5	100·8	<0·0001
Pro:K (g/mEq)†	0·94	0·09	1·11	0·03	1·22	0·03	1·33	0·04	1·54	0·13	
Nutrient intake‡											
Protein (g/d)	59·5	9·8	60·8	8·1	60·5	8·4	59·8	9·3	58·0	8·1	0·028
P (mg/d)	2492	468	2151	310	1949	270	1761	287	1504	273	<0·0001
Food intake (g/d)‡											
Meats	48·3	23·3	58·7	28·1	63·0	29·4	64·4	33·3	65·8	39·4	<0·0001
Fish and shellfish	50·2	28·7	52·1	28·0	51·9	27·4	51·4	30·1	45·8	28·3	0·088
Eggs	33·6	21·5	33·9	19·3	36·7	22·1	36·1	19·9	37·8	23·7	0·019
Dairy products	173·9	147·3	186·4	140·9	152·1	132·3	125·9	102·4	89·5	75·0	<0·0001
Fruits	95·0	87·5	64·6	47·2	53·0	44·2	48·1	41·6	28·8	22·4	<0·0001
Vegetables	308·4	163·9	232·8	110·7	195·6	79·6	170·2	78·6	127·8	58·2	<0·0001
Cereals	348·4	92·4	362·5	78·2	374·3	89·8	395·0	86·4	421·4	95·3	<0·0001

Pro:K, ratio of dietary protein to K.
* A linear trend test was used with the median value in each quintile as a continuous variable in linear regression.
† Calculated using crude nutrient intake values and then energy-adjusted using the residual method.
‡ Energy-adjusted using the residual method.

significantly associated with both PRAL and Pro:K in the present study, the finding on blood pressure may be more reliable than those on other cardiometabolic risk factors that are significantly associated with only one, either PRAL or Pro:K (i.e. total and HDL-cholesterol and BMI and waist circumference). Although both PRAL and Pro:K are established and reasonably valid measures of dietary acid–base load[17], further methodological research may be helpful for clarifying which is the better indicator of diet-induced acid–base load.

Both PRAL and Pro:K were independently and positively associated with systolic and diastolic blood pressure in the present study. Several previous experimental studies have also indicated a significant rise in blood pressure caused by mild metabolic acidosis[6–8], which can be caused by diet[3–5]. Although not established, several plausible mechanisms have been proposed, including increased cortisol production[3] increased Ca excretion[9,10] and reduced citrate excretion[11]. Further research to elucidate the apparent influence of dietary acid–base load on blood pressure using a range of free-living populations is required.

We found an independent and positive association of total and HDL-cholesterol with PRAL, but not with Pro:K. Likewise, an independent and positive association was also found between BMI and waist circumference and Pro:K, but not with PRAL. No significant association with any of the other cardiometabolic risk factors was observed, including fasting TAG, fasting glucose, and glycated Hb. Increasing cortisol production caused by mild metabolic acidosis[3–5] might have a detrimental influence on cardiometabolic risk factors[12–14]. Further investigation of this poorly understood field is warranted.

In the present study, PRAL showed a strong negative association with K (as well as Ca and Mg), but a weak association with protein and P. For foods, the negative association with fruits and vegetables was quite strong compared with that for other foods. Similar trends were observed for Pro:K. Thus, in Japanese populations, dietary acid–base load may be primarily determined by foods rich in K such as fruits and vegetables. This is somewhat different from Western populations, where PRAL was strongly associated not only negatively with fruits and vegetables, but also positively with meats[21].

Mean PRAL and Pro:K were much more acidic in this study of young Japanese women (10·4 mEq/d and 1·2 g/mEq, respectively) than in other two studies of British middle-aged women (3·7 and −7·6 mEq/d and 1·0 and 1·0 g/mEq, respectively)[19,21]. The intake of K, Ca, and Mg as well as

British Journal of Nutrition

Table 4. Cardiometabolic risk factors according to quintile of measures of dietary acid–base load (*n* 1136)*
(Mean values with their standard errors)

	Quintile of measures of dietary acid–base load										
	1 (*n* 227)		2 (*n* 227)		3 (*n* 228)		4 (*n* 227)		5 (*n* 227)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P* for trend†
Potential renal acid load (mEq/d)‡	1·3		7·8		11·2		14·0		18·7		
BMI (kg/m²)§	21·1	0·2	21·2	0·2	21·5	0·2	21·6	0·2	21·4	0·2	0·099
Waist circumference (cm)§,‖	72·7	0·3	72·4	0·3	72·8	0·3	73·3	0·3	73·2	0·3	0·058
Systolic blood pressure (mmHg)§,‖,¶	105·2	0·6	106·5	0·6	106·4	0·6	106·4	0·6	107·3	0·6	0·028
Diastolic blood pressure (mmHg)§,‖,¶	68·1	0·5	69·8	0·5	69·5	0·5	69·5	0·5	69·7	0·5	0·035
Total cholesterol (mg/l)§,‖,¶	1866	21	1854	21	1924	21	1874	21	1925	21	0·042
HDL-cholesterol (mg/l)§,‖,¶	706	8	696	8	711	8	708	8	707	8	0·68
LDL-cholesterol (mg/l)§,‖,¶	1043	18	1048	18	1097	18	1056	18	1103	18	0·021
Fasting TAG (mg/l)§,‖,¶	603	19	614	19	606	19	610	19	622	19	0·56
Fasting glucose (mg/l)§,‖,¶	841	4	837	4	847	4	839	4	838	4	0·76
Glycated Hb (%)§,‖,¶	4·85	0·02	4·88	0·02	4·88	0·02	4·85	0·02	4·86	0·02	0·99
Pro:K (g/mEq)‡	0·96		1·11		1·22		1·33		1·51		
BMI (kg/m²)§	21·0	0·2	21·2	0·2	21·4	0·2	21·6	0·2	21·5	0·2	0·024
Waist circumference (cm)§,‖	72·6	0·3	72·5	0·3	72·7	0·3	73·2	0·3	73·4	0·3	0·012
Systolic blood pressure (mmHg)§,‖,¶	104·7	0·6	106·8	0·6	106·7	0·6	106·6	0·6	107·2	0·6	0·012
Diastolic blood pressure (mmHg)§,‖,¶	67·9	0·5	70·0	0·5	69·2	0·5	69·5	0·5	70·2	0·5	0·009
Total cholesterol (mg/l)§,‖,¶	1878	21	1906	21	1877	21	1907	21	1876	21	0·93
HDL-cholesterol (mg/l)§,‖,¶	712	8	707	8	705	8	711	8	693	8	0·19
LDL-cholesterol (mg/l)§,‖,¶	1049	18	1080	18	1060	18	1086	18	1072	18	0·38
Fasting TAG (mg/l)§,‖,¶	589	19	650	19	593	19	598	19	624	19	0·66
Fasting glucose (mg/l)§,‖,¶	840	4	839	4	840	4	841	4	842	4	0·75
Glycated Hb (%)§,‖,¶	4·87	0·02	4·87	0·02	4·88	0·02	4·88	0·02	4·84	0·02	0·31

Pro:K, ratio of dietary protein to K.

* *n* 1121 for cholesterol (total, HDL, and LDL) and glycated Hb (224 in the first, second, fourth, and fifth and 225 in the third quintiles); 1088 for fasting TAG (217 in the first and fifth and 218 in the second, third, and fourth quintiles); and 1089 for fasting glucose (217 in the first and 218 in the second, third, fourth, and fifth quintiles). For potential renal acid load, median value in each quintile is almost the same (within <0·1 mEq/d difference) in all analyses; for pro:K, median value in each quintile is the same.

† A linear trend test was used with the median value in each quintile as a continuous variable in linear regression.

‡ Calculated using crude nutrient intake values and then energy-adjusted using the residual method. Values are median.

§ Adjusted for residential block (north (Kanto, Hokkaido, and Tohoku), central (Tokai, Hokuriku, and Kinki), or south (Kyushu and Chugoku)), size of residential area (city with population ≥1 million, city with population with <1 million, or town and village), survey year (2006 or 2007), current smoking (yes or no), and physical activity (total metabolic equivalent-h/d, continuous).

‖ Additionally adjusted for BMI (kg/m², continuous).

¶ Additionally adjusted for waist circumference (cm, continuous).

NS British Journal of Nutrition

P was 37–50 % lower, although protein intake was 27 % lower in the Japanese subjects than in the British population[21], which seems to explain the more acidic dietary acid–base load in the present study.

Several limitations of the present study warrant mention. First, the cross-sectional nature of the study does not permit the assessment of causality owing to the uncertain temporality of the association. Nevertheless, there are biologically plausible mechanisms for the relationship between dietary acid–base load and the cardiometabolic risk factors, particularly blood pressure[1,2,6–11], although while the premise for the hypothesis examined is that the acidosis is associated with increased cortisol production[3–5], no measurements for cortisol are unfortunately available, which would help back up findings. Second, our subjects were selected female dietetic students, not a random sample of Japanese women. In addition, because of our recruitment procedure, the exact response rate was unknown, which might have produced recruitment bias. Thus, these results may not apply to the general Japanese population, although our population was on average comparable with a representative sample of Japanese women with similar age range (18–29 years for dietary intake and 20–29 years for cardiometabolic risk factors), at least with regard to the intake of energy (mean: 7117 kJ/d), protein (64·0 g/d), P (894 mg/d), K (1965 mg/d), Ca (459 mg/d), and Mg (210 mg/d) and several cardiometabolic risk factors including BMI (20·9 kg/m^2), systolic blood pressure (108·8 mmHg), diastolic blood pressure (67·0 mmHg), total cholesterol (1806 mg/l), HDL-cholesterol (689 mg/l), and glycated Hb (4·91 %) (data not available for other cardiometabolic risk factors)[34]. Further, because the study population consisted of generally healthy persons, the clinical relevance of our findings remains to be elucidated. Nevertheless, our results should provide valuable insight from a prevention perspective. Third, as with other studies on dietary acid–base load[18,20,21], a semi-quantitative dietary assessment questionnaire (i.e. DHQ) was used to collect dietary data[23–25]. As actual dietary habits were not observed, and the relative validity of the PRAL and Pro:K values derived from the DHQ against the 16 d weighed dietary records was also modest, the results should be interpreted with caution. It should be noted, however, at least for conventional nutrients, that the applicability of the DHQ is comparable to that of dietary questionnaires used in previous studies, in which the relative validity of PRAL and Pro:K was not examined[18,20,21]. Additionally, the misreporting of dietary intake, particularly by overweight subjects, is a serious problem associated with self-report dietary assessment methods[35]. However, at least for dietary protein, K, and Na, BMI-dependent misreporting seems to be cancelled by energy-adjustment[36]. To minimize the influence of dietary misreporting as much as possible[36], we used energy-adjusted values[27]. Actually, the ratio of reported energy intake to estimated basal metabolic rate, a surrogate measure of the general quality of dietary data[35] was not associated with PRAL or Pro:K (data not shown), which suggests that it is unlikely that dietary underreporting had a major effect on the observed associations. Further, because of the lack of a reliable composition table for dietary supplements in Japan, nutrient intake from dietary supplements was not included in the analysis. However, the percentage of subjects who consumed dietary supplements containing mostly protein and minerals during the preceding month was only about 2 %, and the exclusion of these supplement users did not change the results materially (data not shown). This suggests that it is unlikely that dietary supplements had a major effect on the findings. Moreover, although adjustments were attempted to compensate for a variety of potential confounding variables, residual confounding could not be ruled out. In particular, physical activity was assessed relatively roughly from only five activities, which may not have been sufficient. Finally, because the associations between diet-dependent acid load and cardiometabolic risk factors were examined using PRAL and Pro:K estimated from dietary intake, it is not known whether the observed associations were caused by a change in acid–base balance due to dietary intake or by some other mechanism. Additionally, the observed associations between PRAL and Pro:K and cardiometabolic risk factors may be simply due to the effects on cardiometabolic risk factors of intakes of some nutrients or foods associated with PRAL and Pro:K. For example, K and vegetable intake, of which not only the favourable effect on blood pressure is broadly recognized[37–39] but which was also most strongly correlated with PRAL and Pro:K in the present study (Pearson correlation coefficients with PRAL or Pro:K = −0·50 to −0·74), was negatively associated with BMI and waist circumference (P for trend ≤ 0·001), although there was no association with blood pressure (systolic and diastolic) or cholesterol (total and LDL). However, it is not possible to understand what food sources or nutrients are driving the observed associations between dietary acid–base load and cardiometabolic risk factors, because dietary acid–base load used in the present study was derived from estimates of dietary intakes, and hence was associated with these dietary intakes. Thus the results of the present study should be cautiously interpreted without oversimplification.

In conclusion, after adjusting for possible confounding factors, we found that higher PRAL and Pro:K (more acidic dietary acid–base loads) were associated with higher systolic and diastolic blood pressure in free-living young Japanese women. We also found independent positive associations between PRAL and total and LDL-cholesterol, as well as between Pro:K and BMI and waist circumference. Because the cross-sectional nature of the present study does not allow causal inferences, any firm conclusions regarding the effects of dietary acid–base load on cardiometabolic risk factors require additional observational and experimental studies.

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data, and wrote the manuscript. S. S. is a principal researcher of this project and contributed to the concept and design of the study, the study protocol, and data collection and management, and the writing and editing of the manuscript. Y. T. and K. U. contributed to the concept and design of the study, the study protocol, and data collection. All authors contributed to the preparation of the manuscript and approved the final version submitted for publication.

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Dietary glycemic index is associated with decreased premenstrual symptoms in young Japanese women

Kentaro Murakami, M.Sc.[a], Satoshi Sasaki, M.D., Ph.D.[a,*], Yoshiko Takahashi, Ph.D.[b], Kazuhiro Uenishi, Ph.D.[c], Tomoko Watanabe, Ph.D.[d], Toshiyuki Kohri, Ph.D.[e], Mitsuyo Yamasaki, Ph.D.[f], Reiko Watanabe, Ph.D.[g], Keiko Baba, Ph.D.[h], Katsumi Shibata, Ph.D.[i], Toru Takahashi, Ph.D.[j], Hitomi Hayabuchi, Ph.D.[k], Kazuko Ohki, Ph.D.[l], and Junko Suzuki, M.Ed.[m]

[a] *Department of Social and Preventive Epidemiology, School of Public Health, University of Tokyo, Tokyo, Japan*
[b] *Department of Health and Nutrition, School of Home Economics, Wayo Women's University, Chiba, Japan*
[c] *Laboratory of Physiological Nutrition, Kagawa Nutrition University, Saitama, Japan*
[d] *Department of Nutrition, Chiba College of Health Science, Chiba, Japan*
[e] *Department of Food Science and Nutrition, School of Agriculture, Kinki University, Nara, Japan*
[f] *Department of Health and Nutrition Science, Faculty of Health and Social Welfare Science, Nishikyushu University, Saga, Japan*
[g] *Course of Food and Nutrition, Department of Human Life Environments, Niigata Women's College, Niigata, Japan*
[h] *Department of Nutrition, Mie Chukyo University Junior College, Mie, Japan*
[i] *Laboratories of Food Science and Nutrition, Department of Life Style Studies, School of Human Cultures, The University of Shiga Prefecture, Shiga, Japan*
[j] *Graduate School of Human Life Science, Mimasaka University, Okayama, Japan*
[k] *Department of Human Environmental Science, Fukuoka Women's University, Fukuoka, Japan*
[l] *Graduate School of Science for Living System, Showa Women's University, Tokyo, Japan*
[m] *Department of Health and Nutrition, Faculty of Human Sciences, Hokkaido Bunkyo University, Hokkaido, Japan*

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Abstract

Objectives: High glycemic index (GI) carbohydrates may increase brain serotonin, which in turn acts to alleviate premenstrual symptoms, because, although the main determinant of brain serotonin concentration is a high plasma ratio of tryptophan to other large neutral amino acids, a high-GI diet has been shown to increase this ratio. In this observational cross-sectional study, we investigated associations between dietary GI and other dietary carbohydrates and premenstrual symptoms.

Methods: Subjects were 640 female Japanese dietetic students 18–22 y of age. Dietary carbohydrates were assessed using a validated, self-administered, comprehensive diet history questionnaire. Menstrual cycle symptoms were assessed using the retrospective version of the Moos Menstrual Distress Questionnaire (MDQ). Independent associations of dietary GI and glycemic load and intake of available carbohydrate and dietary fiber with the MDQ total score and subscale scores (pain, concentration, behavioral change, autonomic reactions, water retention, and negative affect) in the premenstrual phase (expressed as percentages relative to those in the intermenstrual phase) were examined.

Results: Dietary GI was independently inversely associated with total MDQ score in the premenstrual phase (P for trend = 0.02). Dietary GI also showed independent and inverse associations with several MDQ subscale scores in the premenstrual phase, including concentration, autonomic reactions, and water retention (P for trend < 0.05). Conversely, dietary glycemic load and intake of available carbohydrate and dietary fiber were not associated with any of the MDQ scores in the premenstrual phase.

Conclusion: Dietary GI was independently associated with decreased premenstrual symptoms in a group of young Japanese women.

Keywords: Dietary glycemic index; Dietary carbohydrates; Premenstrual symptoms; Japanese; Epidemiology

* Corresponding author. Tel.: +81-3-5841-7872; fax: +81-3-5841-7873.
E-mail address: stssasak@m.u-tokyo.ac.jp (S. Sasaki).

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Introduction

Premenstrual symptoms are characterized by a set of behavioral, somatic, and affective symptoms of varying severity that occur during the 7–10 d before the onset of menstruation and subside after the beginning of the menstrual flow. Although the etiology of premenstrual symptoms is largely unknown, current evidence suggests that they may arise from a decrease in brain serotonin neurotransmission [1–3]. Although the main determinant of brain serotonin concentration is a high plasma ratio of tryptophan to other large neutral amino acids, high glycemic index (GI) carbohydrates have been shown to increase this ratio [4–6]. Thus, a high-GI diet might be expected to alleviate premenstrual symptoms. Several studies have suggested the favorable effects on premenstrual symptoms of foods with a high GI, such as carbohydrate-rich beverages [7,8] and cereal/potatoes/starches [9]. To our knowledge, however, the relation between dietary GI and premenstrual symptoms has not been investigated. In this study, we cross-sectionally examined the association of dietary GI and other dietary carbohydrate variables, including dietary glycemic load (GL) and intake of available carbohydrate and dietary fiber, with premenstrual symptoms in young Japanese women.

Materials and methods

Subjects

The present study was based on a cross-sectional multicenter survey conducted from January to March 2007 among female dietetic students from 11 institutions in Japan. All measurements at each institution were conducted according to the survey protocol. Briefly, staff at each institution explained an outline of the survey to potential subjects. Those responding positively were then provided detailed written and oral explanations of the survey's general purpose and procedure. The protocol of the study was approved by the ethics committee of the National Institute of Health and Nutrition, and written informed consent was obtained from each subject and from a parent for subjects <20 y of age.

A total of 702 Japanese women took part. For the present analysis, women 18–22 y of age were selected (n = 687). We then excluded women not completing the survey questionnaires (n = 1), those not completing anthropometric measurements (n = 2), those who had been pregnant at any time in the preceding year (n = 3), those with diagnosed endometabolic diseases such as diabetes and thyroid diseases (n = 4), those currently taking oral contraceptives (n = 7) or steroid hormones (n = 16), those who had few or no menstruations during the preceding year (n = 9), those currently receiving dietary counseling from a doctor or dietitian (n = 7), and those with extremely low or high reported energy intakes (<500 or >4000 kcal/d; n = 2). Because some women fell into more than one exclusion category, the final sample comprised 640 women.

Dietary assessment

Dietary habits during the preceding month were assessed using a self-administered diet history questionnaire (DHQ) [10–13]. Responses to the DHQ and to a lifestyle questionnaire were checked at least twice for completeness by the staff (mostly registered dietitians) trained for this survey on the day when other measurements (e.g., body height and weight) were conducted (usually 1–3 d after completion of the questionnaires). When necessary, forms were reviewed with the subject to ensure the clarity of answers. The DHQ is a 16-page structured questionnaire that consists of the following seven sections: general dietary behavior; major cooking methods; consumption frequency and amount of six alcoholic beverages; consumption frequency and semiquantitative portion size of 118 selected food and nonalcoholic beverage items; dietary supplements; consumption frequency and semiquantitative portion size of 19 cereals (rice, bread, and noodles), soup consumed with noodles, and *miso* (fermented soybean paste) soup; and open-ended items for foods consumed regularly (at least once per week) but not appearing in the DHQ [10]. The food and beverage items were selected as foods commonly consumed in Japan, mainly from a food list used in the National Nutrition Survey of Japan, and standard portion sizes were derived mainly from several recipe books for Japanese dishes [10].

Estimates of dietary intake for a total of 150 food items (including five seasonings), energy, and selected nutrients were calculated using an ad hoc computer algorithm for the DHQ [10], which was based on the Standard Tables of Food Composition in Japan [14]. Information on dietary supplements and data from the open-ended questionnaire items were not used in the calculation of dietary intake [10]. Dietary fiber was determined by an enzymatic-gravimetric procedure (modified Prosky's method) [14]. Available carbohydrate was calculated as total carbohydrate minus dietary fiber [15]. Nutrient intake was energy adjusted using the density method, i.e., percentage of energy for macronutrients and amount per 1000 kcal for other nutrients.

Dietary GI was calculated by multiplying the contribution of each food to the daily intake of available carbohydrate by the food's GI value and then summing the products, and dietary GL by multiplying the dietary GI by total daily available carbohydrate intake (divided by 100). These calculation methods are described in detail elsewhere [13,16,17]. Glucose was used as the reference (GI for glucose = 100). Although there have been concerns regarding the utility of the GI for mixed meals (overall diet) [18,19], many researchers have shown that the GI of a mixed meal can be predicted consistently as the weighted mean of the GI value of each component food [20–22]. We used crude values for dietary GI and energy-adjusted values by the

density method (per 1000 kcal) for dietary GL because, by definition, dietary GI is a measurement of carbohydrate quality, not quantity, whereas dietary GL is a measurement of the combination of carbohydrate quality and quantity [16,17].

Detailed description of the validity of the DHQ and the methods used to calculate dietary intake have been published elsewhere [10–13]. For example, Pearson's correlation coefficients between the DHQ and 3-d estimated dietary records in 47 women 38–69 y of age were 0.48 for protein, 0.55 for fat, and 0.48 for total carbohydrate [10]. In addition, Pearson's correlation coefficients between the DHQ and 16-d weighed dietary records in 92 women 31–69 y of age were 0.62 for available carbohydrate, 0.50 for dietary GI, and 0.57 for dietary GL [13] and 0.47 for protein, 0.60 for fat, 0.64 for total carbohydrate, and 0.71 for dietary fiber (S. Sasaki, unpublished observations, 2007).

Assessment of premenstrual symptoms

Menstrual cycle symptoms during the preceding year were assessed using the Japanese version [9] of the modification by Magos et al. [23] of the retrospective version of the Moos Menstrual Distress Questionnaire (MDQ) [24]. The MDQ, incorporated into the lifestyle questionnaire, consists of a total of 45 symptom items [23], which are grouped into eight subscales [24]: pain, concentration, behavioral change, autonomic reactions, water retention, negative affect, arousal, and non-specific adverse symptoms designed to detect those experiencing symptoms (control). Each symptom item was rated by each subject on a 5-point scale from 1 (no experience of the symptom) to 5 (disabling or incapacitating experience of the symptom) [9], separately for the three menstrual cycle phases (menstrual, during menstrual flow; premenstrual, the week before the beginning of menstrual flow; and intermenstrual, remainder of cycle) [24]. The MDQ scores were calculated for each subscale and the total score (excluding arousal and control) for each cycle phase [9,25]. The total and subscale MDQ scores in the premenstrual phase expressed as percentages relative to those in the intermenstrual phase were used in the present study.

Assessment of other variables

Body height was measured to the nearest 0.1 cm with the subject standing without shoes. Body weight in light indoor clothes was measured to the nearest 0.1 kg. Body mass index was calculated as body weight (kilograms) divided by the square of body height (meters). In the lifestyle questionnaire, the subject reported her residential area, which was grouped into one of three regions (residential block: north [Kanto, Hokkaido, and Tohoku], central [Kinki, Tokai, and Hokuriku], or south [Kyushu and Chugoku]). The residential areas were also grouped into three categories according to population size (size of residential area: city with population ≥1 million, city with population <1 million, or town and village). Current smoking (yes or no), age at menarche, whether currently experiencing menstrual flow, date of the start of the most recent (or current) menstrual flow, usual length of the menstrual cycle, and usual number of days of bleeding were also self-reported in the lifestyle questionnaire. For women who reported irregular menstrual cycles, we asked the range of the length of cycles and allotted the median as the cycle length [26]. According to information on whether the subject was currently menstruating, the date of the start of the most recent menstruation, the usual length of the menstrual cycle, and the date the lifestyle questionnaire was completed, the subjects were divided into three categories of menstrual cycle phase as at the time of the study (menstrual, premenstrual, or intermenstrual). Physical activity was calculated as the average metabolic equivalent-hours per day [27] on the basis of the frequency and duration of five different activities (sleeping, high- and moderate-intensity activities, walking, and sedentary activities) over the preceding month, as reported in the lifestyle questionnaire.

Statistical analysis

All statistical analyses were performed using SAS 8.2 (SAS Institute, Cary, NC, USA). Linear regression models were constructed using the PROC GLM procedure to examine the association between dietary carbohydrates, i.e., available carbohydrate and dietary fiber intake and dietary GI and GL and premenstrual symptoms, i.e., total and subscale MDQ scores in the premenstrual phase. For these analyses, subjects were categorized into quintiles according to dietary carbohydrates. Mean ± SE premenstrual symptom scores in the premenstrual phase were calculated by quintiles of dietary carbohydrates after multivariate adjustment for potential confounding factors, which included age, body mass index, residential block, size of residential area, current smoking, age at menarche, usual length of the menstrual cycle, usual number of days of bleeding, menstrual cycle phase at the time of the study, and physical activity. For each dietary carbohydrate variable, dietary intakes of other nutrients were also added as confounding factors (protein and dietary fiber for available carbohydrate; protein and fat for dietary fiber; and protein, fat, and dietary fiber for dietary GI and GL, respectively). Fat and available carbohydrate were not added simultaneously in the model because of their strong correlation (Pearson's correlation coefficient −0.91); inclusion of available carbohydrate instead of fat as a confounding factor did not materially change the results of dietary fiber, GI, and GL (data not shown). Because alcohol intake was extremely low (mean 0.6% energy), it was not considered as a confounding factor. Marital status, self-reported in the lifestyle questionnaire, was also not considered as a confounding factor because there was only one married woman. Linear trends with increasing levels of dietary carbohydrates were tested by assigning

each participant a median value for the category and modeling this value as a continuous variable. All reported P values are two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Subject characteristics are presented in Table 1. Mean total MDQ score in the premenstrual phase (relative to that in the intermenstrual phase) was 125.7%. Mean intakes of protein, fat, and available carbohydrate were 13.2%, 28.9%, and 53.5% energy, respectively. Mean dietary fiber intake was 6.6 g/1000 kcal, and mean dietary GI and GL were 65.4 and 83.0/1000 kcal, respectively. White rice was the major contributor to dietary GI and GL (46.6%). Subject characteristics according to quintile of total MDQ score in the premenstrual phase are also listed in Table 1. There were more current smokers in the higher quintiles of total MDQ score in the premenstrual phase. Women in the higher quin-

Table 1
Subject characteristics according to quintile of MDQ total score in the premenstrual phase*

Variable	All (n = 640)	Quintile of MDQ total score in premenstrual phase					$P^{\dagger}$
		1 (n = 122)	2 (n = 135)	3 (n = 128)	4 (n = 127)	5 (n = 128)	
MDQ total score in premenstrual phase (%)	125.7 ± 34.2	99.6 ± 2.1	106.1 ± 2.4	114.4 ± 2.7	128.7 ± 5.8	179.3 ± 42.0	<0.0001
MDQ subscale scores in premenstrual phase (%)							
Pain	132.7 ± 41.6	99.5 ± 6.3	114.8 ± 15.3	127.2 ± 24.2	145.8 ± 36.0	175.7 ± 55.4	<0.0001
Concentration	114.1 ± 33.7	99.5 ± 3.6	100.7 ± 3.7	102.6 ± 8.6	112.0 ± 18.3	155.9 ± 54.7	<0.0001
Behavioral change	129.4 ± 52.1	100.4 ± 3.1	103.4 ± 9.8	111.5 ± 17.4	131.2 ± 27.1	200.5 ± 74.6	<0.0001
Autonomic reactions	106.9 ± 25.8	99.2 ± 6.0	99.9 ± 1.7	102.9 ± 13.1	105.6 ± 15.4	126.8 ± 48.7	<0.0001
Water retention	143.6 ± 58.5	102.2 ± 17.3	118.5 ± 21.1	137.9 ± 34.8	148.9 ± 43.1	209.8 ± 81.3	<0.0001
Negative affect	130.4 ± 54.6	99.6 ± 3.5	103.1 ± 8.2	111.2 ± 16.0	130.9 ± 28.4	207.4 ± 76.2	<0.0001
Age (y)	19.7 ± 1.1	19.5 ± 1.1	19.6 ± 1.0	19.6 ± 1.0	19.8 ± 1.1	19.9 ± 1.2	0.010
Body height (cm)	158.5 ± 5.4	159.2 ± 5.1	158.2 ± 6.1	158.5 ± 5.2	158.2 ± 5.4	158.6 ± 5.4	0.84
Body weight (kg)	53.7 ± 7.4	55.2 ± 8.1	53.6 ± 6.9	53.9 ± 7.3	52.8 ± 6.8	53.3 ± 7.6	0.14
Body mass index (kg/m^2)	21.4 ± 2.5	21.7 ± 2.7	21.4 ± 2.5	21.4 ± 2.4	21.1 ± 2.4	21.2 ± 2.6	0.10
Residential block							0.96
North (Kanto, Hakkaido, and Tohoku)	349 (55)	66 (54)	69 (51)	73 (57)	70 (55)	71 (55)	
Central (Kinki, Tokai, and Hokuriku)	179 (28)	38 (31)	42 (31)	31 (24)	33 (26)	35 (27)	
South (Kyushu and Chugoku)	112 (18)	18 (15)	24 (18)	24 (19)	24 (19)	22 (17)	
Size of residential area							0.42
City with population ≥1 million	98 (15)	19 (16)	23 (17)	20 (16)	16 (13)	20 (16)	
City with population <1 million	514 (80)	97 (80)	109 (81)	102 (80)	107 (84)	99 (77)	
Town and village	28 (4)	6 (5)	3 (2)	6 (5)	4 (3)	9 (7)	
Current smoking							0.008
No	626 (98)	122 (100)	133 (99)	129 (98)	123 (99)	122 (95)	
Yes	14 (2)	0 (0)	2 (1)	2 (2)	4 (3)	6 (5)	
Age at menarche (y)	12.3 ± 1.4	12.6 ± 1.4	12.5 ± 1.6	12.3 ± 1.4	12.2 ± 1.3	12.0 ± 1.5	0.002
Usual length of menstrual cycle (d)	31.8 ± 11.1	32.9 ± 13.3	30.5 ± 7.5	31.6 ± 10.3	32.8 ± 11.7	31.5 ± 11.9	0.90
Usual number of days of bleeding	6.1 ± 1.2	6.1 ± 1.3	6.3 ± 1.1	6.1 ± 1.1	6.1 ± 1.2	6.2 ± 1.4	0.99
Menstrual cycle phase at time of study							0.30
Menstrual phase	122 (19)	23 (19)	32 (24)	21 (16)	25 (20)	21 (16)	
Premenstrual phase	179 (28)	35 (29)	37 (27)	40 (31)	31 (24)	36 (28)	
Intermenstrual phase	339 (53)	64 (52)	66 (49)	67 (52)	71 (56)	71 (55)	
Physical activity (total metabolic equivalent-hours/d)	33.8 ± 2.8	33.7 ± 2.0	34.3 ± 3.9	33.8 ± 2.8	33.6 ± 2.3	33.7 ± 2.3	0.42
Protein intake (% energy)	13.2 ± 1.8	13.1 ± 1.7	13.3 ± 1.8	13.0 ± 1.8	13.2 ± 1.6	13.4 ± 2.2	0.20
Fat intake (% energy)	28.9 ± 5.2	28.9 ± 5.4	28.5 ± 5.5	28.8 ± 5.0	29.3 ± 5.4	29.1 ± 4.6	0.44
Available carbohydrate intake (% energy)[‡]	53.5 ± 6.0	53.8 ± 6.0	54.1 ± 6.4	53.6 ± 6.3	53.2 ± 5.8	52.9 ± 5.5	0.12
Dietary fiber intake (g/1000 kcal)	6.6 ± 1.8	6.7 ± 1.8	6.6 ± 1.9	6.6 ± 1.9	6.5 ± 1.7	6.7 ± 1.7	0.94
Dietary glycemic index[§]	65.4 ± 4.0	65.9 ± 3.8	65.7 ± 3.9	65.6 ± 4.0	64.9 ± 4.1	65.1 ± 4.1	0.094
Dietary glycemic load (/1000 kcal)[§]	83.0 ± 12.7	83.9 ± 12.4	84.4 ± 13.4	83.4 ± 13.7	81.8 ± 11.6	81.5 ± 12.2	0.045

MDQ, Menstrual Distress Questionnaire

* Values are means ± SDs or number of subjects (percentages). MDQ scores in the premenstrual phase were expressed as percentages relative to those in the intermenstrual phase.

† For continuous variables, a linear trend test was used, with the median value in each quintile as a continuous variable in linear regression; for categorical variables, a Mantel-Haenszel chi-square test was used.

‡ Calculated as total carbohydrate minus dietary fiber.

§ Glycemic index for glucose = 100.

tiles of total MDQ score in the premenstrual phase had a higher mean age and lower means for age at menarche and dietary GL. Independent associations between dietary carbohydrates and MDQ scores in the premenstrual phase are presented in Table 2. After adjustment for potential confounding factors, dietary GI was negatively associated with total MDQ score in the premenstrual phase (mean difference between lowest and highest quintiles −11.8%, P for trend = 0.016). Dietary GI also showed independent and negative associations with several MDQ subscale scores in the premenstrual phase, including concentration (mean difference −9.7%, P for trend = 0.042), autonomic reactions (mean difference −6.8%, P for trend = 0.045), and water retention (mean difference −18.6%, P for trend = 0.024).

Table 2
MDQ scores in the premenstrual phase according to quintile of each dietary carbohydrate variable (n = 640)*

Variable	Quintile of each dietary carbohydrate variable					P for trend†
	1 (n = 128)	2 (n = 128)	3 (n = 128)	4 (n = 128)	5 (n = 128)	
Available carbohydrate intake (% energy)‡	46.0	50.5	53.5	56.8	61.1	
MDQ total score in premenstrual phase (%)§‖	126.4 ± 3.3	121.9 ± 3.1	128.8 ± 3.0	128.8 ± 3.1	122.4 ± 3.3	0.86
MDQ subscale scores in premenstrual phase (%)§‖						
Pain	135.0 ± 4.0	129.7 ± 3.7	134.7 ± 3.6	136.3 ± 3.7	127.8 ± 4.0	0.52
Concentration	119.3 ± 3.2	108.3 ± 3.0	120.1 ± 3.0	114.1 ± 3.0	108.8 ± 3.3	0.14
Behavioral change	126.9 ± 5.0	123.1 ± 4.6	136.0 ± 4.6	132.4 ± 4.7	128.6 ± 5.1	0.51
Autonomic reactions	108.1 ± 2.5	105.4 ± 2.4	105.7 ± 2.3	111.4 ± 2.3	103.7 ± 2.5	0.69
Water retention	142.5 ± 5.6	145.6 ± 5.2	147.9 ± 5.1	149.3 ± 5.2	132.5 ± 5.6	0.40
Negative affect	129.2 ± 5.3	126.6 ± 5.0	131.9 ± 4.9	133.3 ± 4.9	131.2 ± 5.3	0.58
Dietary fiber intake (g/1000 kcal)	4.6	5.5	6.4	7.4	9.1	
MDQ total score in premenstrual phase (%)§¶	127.5 ± 3.1	125.1 ± 3.1	123.6 ± 3.0	125.7 ± 3.0	126.4 ± 3.1	0.97
MDQ subscale scores in premenstrual phase (%)§¶						
Pain	134.1 ± 3.8	133.3 ± 3.7	128.4 ± 3.6	132.8 ± 3.7	134.8 ± 3.8	0.81
Concentration	115.8 ± 3.1	115.0 ± 3.1	114.1 ± 3.0	112.7 ± 3.0	113.0 ± 3.1	0.47
Behavioral change	130.9 ± 4.8	134.4 ± 4.7	125.6 ± 4.6	129.6 ± 4.7	126.5 ± 4.8	0.42
Autonomic reactions	106.5 ± 2.4	104.0 ± 2.3	107.5 ± 2.3	106.6 ± 2.3	109.8 ± 2.4	0.22
Water retention	149.7 ± 5.3	136.7 ± 5.2	137.0 ± 5.1	149.8 ± 5.1	144.6 ± 5.3	0.83
Negative affect	132.6 ± 5.0	127.5 ± 4.9	129.3 ± 4.8	129.1 ± 4.9	133.7 ± 5.1	0.73
Dietary glycemic index#	60.5	63.5	65.5	67.6	70.8	
MDQ total score in premenstrual phase (%)§**	132.8 ± 3.1	124.1 ± 3.0	127.8 ± 3.0	122.7 ± 3.0	121.0 ± 3.1	0.016
MDQ subscale scores in premenstrual phase (%)§**						
Pain	139.5 ± 3.8	133.2 ± 3.6	132.1 ± 3.7	129.0 ± 3.7	129.6 ± 3.8	0.061
Concentration	120.5 ± 3.1	112.8 ± 3.0	115.6 ± 3.0	111.1 ± 3.0	110.8 ± 3.1	0.042
Behavioral change	136.5 ± 4.8	126.1 ± 4.6	130.9 ± 4.7	129.8 ± 4.7	123.6 ± 4.8	0.15
Autonomic reactions	111.8 ± 2.4	106.7 ± 2.3	106.9 ± 2.3	103.9 ± 2.3	105.0 ± 2.4	0.045
Water retention	155.9 ± 5.3	139.1 ± 5.1	150.5 ± 5.1	135.0 ± 5.2	137.3 ± 5.3	0.024
Negative affect	137.8 ± 5.0	128.4 ± 4.8	133.6 ± 4.9	128.7 ± 4.9	123.7 ± 5.1	0.092
Dietary glycemic load (/1000 kcal)#	67.2	76.2	82.9	88.6	99.2	
MDQ total score in premenstrual phase (%)§**	128.7 ± 4.7	123.3 ± 3.3	129.8 ± 3.0	126.8 ± 3.2	119.8 ± 4.8	0.46
MDQ subscale scores in premenstrual phase (%)§**						
Pain	132.4 ± 5.7	132.1 ± 4.0	134.7 ± 3.6	135.2 ± 3.9	129.0 ± 5.8	0.87
Concentration	119.0 ± 4.6	109.8 ± 3.3	119.4 ± 3.0	114.7 ± 3.2	107.7 ± 4.7	0.36
Behavioral change	130.1 ± 7.2	124.9 ± 5.1	133.5 ± 4.6	132.0 ± 4.9	126.5 ± 7.4	0.99
Autonomic reactions	106.7 ± 3.6	104.2 ± 2.5	108.3 ± 2.3	110.2 ± 2.5	104.9 ± 3.7	0.87
Water retention	152.2 ± 7.9	148.5 ± 5.7	146.4 ± 5.1	144.1 ± 5.5	126.7 ± 8.2	0.10
Negative affect	136.3 ± 7.5	127.2 ± 5.3	136.7 ± 4.8	128.7 ± 5.2	123.3 ± 7.7	0.43

MDQ, Menstrual Distress Questionnaire

* Values are medians for dietary carbohydrate variables and means ± SEs for MDQ scores. MDQ scores in the premenstrual phase were expressed as percentages relative to those in the intermenstrual phase.

† A linear trend test was used with the median value in each quintile as a continuous variable in linear regression.

‡ Calculated as total carbohydrate minus dietary fiber.

§ Adjusted for age (years, continuous), body mass index (kilograms per square meter, continuous), residential block (north: Kanto, Hokkaido, and Tohoku; central: Kinki, Tokai, and Hokuriku; or south: Kyushu and Chugoku), size of residential area (city with population ≥1 million, city with population with <1 million, or town and village), current smoking (yes or no), age at menarche (years, continuous), usual length of the menstrual cycle (days, continuous), usual number of days of bleeding (continuous), menstrual cycle phase at the time of the study (menstrual, premenstrual, or intermenstrual), and physical activity (total metabolic equivalents-hours per day, continuous).

‖ Also adjusted for intakes (continuous) of protein (percentage of energy) and dietary fiber (grams per 1000 kcal).

¶ Also adjusted for intakes (continuous) of protein (percentage of energy) and fat (percentage of energy).

Glycemic index for glucose = 100.

** Also adjusted for intakes (continuous) of protein (percentage of energy), fat (percentage of energy), and dietary fiber (grams per 1000 kcal).

Conversely, intake of available carbohydrate and dietary fiber and dietary GL were not associated with any of the MDQ scores in the premenstrual phase.

Discussion

In a group of young Japanese women, we found that dietary GI was inversely associated with total MDQ score in the premenstrual phase after adjustment for possible confounding factors. We also found independent inverse associations between dietary GI and several MDQ subscale scores in this phase, including concentration, autonomic reactions, and water retention. Conversely, available carbohydrate and dietary fiber intake and dietary GL were not associated with any of the MDQ scores in the premenstrual phase. To our knowledge, this is the first study to examine the relation between dietary GI and premenstrual symptoms.

The mechanism of the association between a high-GI diet and a decrease in premenstrual symptoms is currently unknown. However, one possibility is that a high-GI diet may increase brain serotonin, which in turn acts to alleviate premenstrual symptoms [1–3], because, although the main determinant of brain serotonin concentration is a high plasma ratio of tryptophan (a precursor of brain serotonin) to other large neutral amino acids, a high-GI diet has been shown to increase this ratio [4–6]. Nevertheless, this is still an area of controversy, because increased plasma levels of tryptophan (in relation to other large neutral amino acids) caused by carbohydrate meals may not necessarily correspond with increased cerebrospinal levels of tryptophan [28].

To our knowledge, only a few studies have assessed the relation between dietary carbohydrates and premenstrual symptoms. In two small intervention studies [7,8], an experimental beverage that contained a mixture of carbohydrates previously shown to significantly raise the circulating tryptophan to large neutral amino acid ratio was shown to alleviate premenstrual symptoms more effectively than another beverage that contained a similar amount of carbohydrates but that had previously been shown to leave the ratio unchanged. In a small cross-sectional Japanese study [9], the intake of cereal/potatoes/starches, the main component of which was white rice [29], a food with a high GI [30–32], was associated with the alleviation of premenstrual symptoms, whereas total carbohydrate intake was not. These results and the potential mechanism described above are closely consistent with our findings of an inverse association of dietary GI, but not of intake of available carbohydrate or dietary fiber, with premenstrual symptoms. We do not know why dietary GL was not associated with premenstrual symptoms in the present study. As recently suggested, dietary GL might provide little information beyond the available carbohydrate content of the diet [13].

Several limitations of the present study warrant mention. First, the cross-sectional nature of the study did not permit the assessment of causality owing to the uncertain temporality of the association, although a biologically plausible mechanism for the relation between dietary GI and premenstrual symptoms has been identified. Second, our subjects were selected female dietetic students, not a random sample of Japanese women. In addition, because of our recruitment procedure, the exact response rate was unknown, which might have produced recruitment bias. Thus, our results might not apply to the general Japanese population, although our population was on average comparable to a representative sample of Japanese women 18–29 y of age, at least with regard to the intake of energy (mean 1771 versus 1701 kcal/d), protein (58.8 versus 64.0 g/d), fat (57.9 versus 56.0 g/d), total carbohydrate (246.2 versus 226.4 g/d), and dietary fiber (11.7 versus 12.0 g/d) [29]. Furthermore, the clinical relevance of our findings is unknown. Nevertheless, our results should provide valuable insight from a prevention perspective. The impact of the high-GI diet may be more evident if the study is replicated in women with more severe premenstrual symptoms (i.e., in women with premenstrual syndrome).

Because dietary data were collected using a self-administered semiquantitative dietary assessment questionnaire (i.e., DHQ) [10–13], actual dietary habits were not observed. In addition, as with other epidemiologic studies on dietary GI and GL, the DHQ is not designed specifically to measure dietary GI and GL [10–13]. The results should thus be interpreted with caution, although the validity of the DHQ appears reasonable with regard to not only commonly studied nutrients [10–12] but also dietary GI and GL [13].

We assessed premenstrual symptoms using a retrospective questionnaire (i.e., MDQ) [24]. Notwithstanding that it is often the only choice in large-scale epidemiologic research, this method has been criticized for providing an inflated estimation of symptom severity and being heavily reliant on subjects' memory of past menstrual-related symptoms. The MDQ is the most widely recognized and used questionnaire, and most of the various other measurement instruments currently available draw on aspects of it. In the present study, the MDQ scores of subscales not varying across the menstrual cycle (i.e., arousal and control) [24] showed a very small fluctuation between the premenstrual and intermenstrual phases (<4%), in contrast to the other MDQ subscale scores (7–44%), which may support the validity of this method.

Dietary habits and menstrual symptoms were evaluated in different periods, namely in the preceding month for the former and in the preceding year for the latter. However, the results did not materially change when analysis was limited to women reporting a stable diet with the preceding year (n = 526; data not shown). As an additional difficulty, dietary intake [33], and possibly answers on dietary habits and menstrual symptoms, may vary across the menstrual cycle. To minimize its influence, we treated menstrual cycle phase at the time of the study as a confounding factor.

Although adjustments were attempted to compensate for a variety of potential confounding variables including age, age at menarche, smoking status, usual length of the menstrual cycle, usual number of days of bleeding, body mass index, and physical activity [9,34,35], residual confounding could not be ruled out. In particular, although the influence of stress on premenstrual symptoms has been suggested [34,35], we unfortunately had no information on stress in the present study. Further research on diet and premenstrual symptoms should take stress into account. Because our sample size was modest, statistical power may have been insufficient to allow the detection of associations between dietary GI and some of the MDQ subscale scores.

Conclusions

We observed independent inverse associations of dietary GI with the MDQ total and several subscale scores (concentration, autonomic reactions, and water retention) in the premenstrual phase in young Japanese women. Because of the cross-sectional nature, any firm conclusions regarding the effects of dietary GI on premenstrual symptoms require additional studies.

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Total n-3 polyunsaturated fatty acid intake is inversely associated with serum C-reactive protein in young Japanese women

Kentaro Murakami[a,b], Satoshi Sasaki[a,c,*], Yoshiko Takahashi[a,d], Kazuhiro Uenishi[e], Mitsuyo Yamasaki[f], Hitomi Hayabuchi[g], Toshinao Goda[h], Jun Oka[i], Keiko Baba[j], Kazuko Ohki[k], Kanako Muramatsu[l], Yoshiko Sugiyama[m]

[a]*Nutritional Epidemiology Program, National Institutes of Health and Nutrition, Tokyo, Japan*
[b]*Department of Epidemiology and International Health, Research Institute, International Medical Center of Japan, Tokyo, Japan*
[c]*Department of Social and Preventive Epidemiology, School of Public Health, The University of Tokyo, Tokyo, Japan*
[d]*Department of Health and Nutrition, School of Home Economics, Wayo Women's University, Chiba, Japan*
[e]*Laboratory of Physiological Nutrition, Kagawa Nutrition University, Saitama, Japan*
[f]*Department of Health and Nutrition Science, Faculty of Health and Social Welfare Science, Nishikyushu University, Saga, Japan*
[g]*Department of Human Environmental Science, Fukuoka Women's University, Fukuoka, Japan*
[h]*Department of Nutrition, School of Food and Nutritional Sciences and COE21, University of Shizuoka, Shizuoka, Japan*
[i]*Department of Food and Nutrition, Faculty of Home Economics, Tokyo Kasei University, Tokyo, Japan*
[j]*Department of Nutrition, Mie Chukyo University Junior College, Mie, Japan*
[k]*Graduate School of Science for Living System, Showa Women's University, Tokyo, Japan*
[l]*Course of Food and Nutrition, Department of Human Life Environments, Niigata Women's College, Niigata, Japan*
[m]*Department of Nutrition Management, Faculty of Health and Nutrition, Minamikyushu University, Miyazaki, Japan*

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Abstract

Little is known about the relation of dietary factors to circulating C-reactive protein (CRP) concentrations in young adults and non-Western populations. We cross-sectionally examined associations between dietary intake and serum CRP concentrations in young Japanese women. The subjects were 443 female Japanese dietetic students aged 18 to 22 years. Dietary intake was assessed with a validated, self-administered, comprehensive, diet history questionnaire. Serum CRP concentrations were measured by highly sensitive nephelometry. The prevalence of elevated CRP (≥1 mg/L) was 5.6%. After adjustment for possible confounding factors including body mass index, a significant inverse association was seen between total n-3 polyunsaturated fatty acid intake and elevated CRP. The multivariate adjusted odds ratios of elevated CRP for women with intake below and above the median (1.1% of energy) were 1.00 and 0.33 (95% confidence interval, 0.13-0.82; $P = .02$), respectively. Intake of eicosapentaenoic acid + docosahexaenoic acid and α-linolenic acid was not associated with elevated CRP concentrations ($P = .62$ and $P = .27$, respectively). Vitamin C intake was independently inversely associated with elevated CRP, although the association was nonsignificant ($P = .10$). No clear associations were observed for other dietary factors examined including total fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, total dietary fiber, soluble dietary fiber, insoluble dietary fiber, and magnesium; fruits, vegetables, and fish and shellfish; and dietary glycemic load ($P = .27$ to $P = .99$). In conclusion, total n-3

* Corresponding author. Department of Social and Preventive Epidemiology, School of Public Health, The University of Tokyo, Tokyo 113-0033, Japan.
E-mail address: stssasak@m.u-tokyo.ac.jp (S. Sasaki).

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polyunsaturated fatty acid intake showed an independent inverse association with elevated serum CRP concentration in a group of young Japanese women.

Keywords: n-3 polyunsaturated fatty acid; C-reactive protein; Diet; Young Japanese women; Epidemiology
Abbreviations: ALA, α-linolenic acid; BMI, body mass index; CRP, C-reactive protein; DHA, docosahexaenoic acid; DHQ, diet history questionnaire; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

1. Introduction

C-reactive protein (CRP) is a sensitive marker of inflammation that is independently and directly associated with cardiovascular disease [1], type 2 diabetes [2], and metabolic syndrome [3]. Identification of modifiable lifestyle factors that influence circulating CRP concentrations (eg, dietary habits) is thus vitally important from a prevention perspective. Nutrients suggested to be associated with CRP concentration in epidemiologic studies include fat and several fatty acids [4-8], dietary fiber [4,9,10], magnesium [11,12], and vitamin C [13,14]. At the food level, associations have been reported for fruits [14-16], vegetables [14-16], and fish [17]. An association with dietary glycemic load (a measure of carbohydrate quality and quantity) has also been suggested [18]. However, almost all these studies have been conducted in middle-aged and elderly populations in Western countries [4-6,9-18], and evidence from non-Western countries [7,8] and young adult populations [7] is extremely limited.

Here, we conducted a cross-sectional study of associations between selected dietary factors, that is, intake of total fat, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), total n-3 PUFA, eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA), α-linolenic acid (ALA), total dietary fiber, soluble dietary fiber, insoluble dietary fiber, magnesium, and vitamin C; intake of fruits, vegetables, and fish and shellfish; and dietary glycemic load, with serum CRP concentration in a group of young Japanese women.

Research among Japanese populations is important because the differences in dietary habits between Japanese and Western populations (particularly, higher intake of fish and, hence, n-3 PUFA in Japanese than in Western populations [19,20]) hamper the extrapolation of findings in Western countries to Japanese. Research among young adult populations is also important from the standpoint of prevention. We hypothesized that dietary habits (particularly n-3 PUFA intake) influence serum CRP concentrations in young Japanese women.

2. Methods and materials

2.1. Subjects

The present study was based on a multicenter survey conducted from February to March 2006 among female dietetic students from 10 institutions in Japan. All measurements at each institution were conducted according to the survey protocol. Staff at each institution provided an outline of the survey to potential subjects. Those responding positively were then provided detailed written and oral explanations of the general purpose and procedure of the survey. The protocol of the study was approved by the Ethics Committee of the National Institutes of Health and Nutrition, and written informed consent was obtained from each subject and also from a parent for subjects younger than 20 years. A total of 474 women took part. We excluded subjects whose CRP concentrations had not been measured (n = 22), those with CRP concentrations of 10 mg/L or greater (n = 2) on the basis that such high concentrations were likely caused by infection or an underlying medical problem not related to diet [21], and those aged younger than 18 years or aged 23 years or older (n = 7). The final sample thus comprised 443 women aged 18 to 22 years. All women were free from diabetes, hypertension, and cardiovascular disease, and all reported energy intakes within the relatively strict range used in our previous study (775-3450 kcal/d) [22].

2.2. Dietary assessment

Dietary habits during the preceding month were assessed using a previously validated self-administered comprehensive diet history questionnaire (DHQ) [23-25]. Responses to the DHQ as well as to an accompanying lifestyle questionnaire were checked at least twice for completeness and reviewed with the subject when necessary to ensure the clarity of answers. Estimates of dietary intake for a total of 150 food and beverage items, energy, and selected nutrients were calculated using an ad hoc computer algorithm for the DHQ based on the *Standard Tables of Food Composition in Japan* [26,27]. Although dietary supplement use was queried in the DHQ, intake from supplements was not included in the calculation because of the lack of a reliable composition table of dietary supplements in Japan. Dietary glycemic load was calculated according to a procedure described elsewhere [28,29]. Pearson correlation coefficients between the DHQ and 3-day estimated dietary records were 0.55 for total fat, 0.75 for SFA, 0.50 for MUFA, 0.37 for PUFA, and 0.45 for vitamin C in 47 women [23]. In addition, Pearson correlation coefficients between the DHQ and serum concentrations were 0.66 for EPA + DHA and 0.36 for ALA in 44 women [25].

2.3. Serum CRP concentrations

About 1 to 3 days after completion of the questionnaires, peripheral blood samples were obtained from subjects after an overnight fast. Blood was collected in evacuated tubes containing no additives, allowed to clot, and centrifuged at 3000*g* for 10 minutes at room temperature to separate the serum. Blood samples were transported at −20 °C to a laboratory in Tokyo (SRL Inc, Tokyo, Japan). Serum CRP concentrations were measured by highly sensitive nephelometry at SRL Inc. In-house quality-control procedures were fulfilled at SRL Inc and showed within- and between-assay coefficients of variation of 3.1% and 2.7%, respectively. The assay is sufficiently sensitive to detect 0.050 mg/L. Undetectable CRP values were recorded as 0.025 mg/L (n = 81). Serum CRP concentrations of 1 mg/L or greater were considered elevated [8,21].

2.4. Other variables

Body height was measured to the nearest 0.1 cm, with the subject standing without shoes. Body weight in light indoor clothes was measured to the nearest 0.1 kg. Body mass index (BMI) was calculated as body weight (in kilograms) divided by the square of body height (in meters). In the lifestyle questionnaire, the subject reported her residential area, which was grouped into 1 of 3 regions (north [Kanto and Tohoku], central [Tokai and Hokuriku], or south [Kyushu and Chugoku]) and also into 3 categories according to population size (city with population of 1 million or greater, city with population of less than 1 million, or town and village). Current smoking status (yes or no) was self-reported in the lifestyle questionnaire. Alcohol drinking was assessed using the DHQ and grouped into 3 categories (nondrinker, >0% to <1% of energy, or ≥1% of energy). Dietary supplement use (yes or no) was assessed as part of the DHQ. Physical activity was computed as average metabolic equivalent hours per day [30] on the basis of the frequency and duration of 5 different activities (sleeping, high- and moderate-intensity activities, walking, and sedentary activities) over the preceding month as reported in the lifestyle questionnaire.

2.5. Statistical analysis

Associations with elevated serum CRP concentration were examined for the intake of selected nutrients, including total fat, SFA, MUFA, PUFA, total n-3 PUFA, EPA + DHA, ALA, total dietary fiber, soluble dietary fiber, insoluble dietary fiber, magnesium, and vitamin C; selected foods, including fruits, vegetables, and fish and shellfish; and dietary glycemic load. We used energy-adjusted values of dietary intake, that is, percentage of energy from total fat and fatty acids and amounts per 4184 kJ of other dietary variables. For analyses, subjects were divided into 2 categories according to the median value of dietary intake. Odds ratios (ORs) and 95% confidence intervals (CIs) for elevated serum CRP concentrations were calculated after multivariate adjustment for potential confounding factors, including residential block, size of residential area, current smoking, alcohol drinking, dietary supplement use, physical activity (continuous), and BMI (continuous). All statistical analyses were performed using SAS statistical software (version 8.2; SAS Institute Inc, Cary, NC). All reported *P* values are 2-tailed, with a *P* value of less than .05 considered statistically significant.

Table 1
Basic characteristics of 443 Japanese women aged 18 to 22 years[a]

	Value
Age (y)	19.5 ± 1.0
Body height (cm)	158.1 ± 5.6
Body weight (kg)	53.3 ± 8.1
BMI (kg/m^2)	21.3 ± 2.9
Serum CRP concentration (mg/L)	0.30 ± 0.73
<1 mg/L	418 (94.4)
≥1 mg/L	25 (5.6)
Residential block	
North (Kanto and Tohoku)	268 (60.5)
Central (Tokai and Hokuriku)	73 (16.5)
South (Kyushu and Chugoku)	102 (23.0)
Size of residential area	
City with population of ≥1 million	77 (17.4)
City with population of <1 million	329 (74.3)
Town and village	37 (8.4)
Current smoking	
No	428 (96.6)
Yes	15 (3.4)
Alcohol drinking	
Nondrinker	262 (59.1)
>0% to <1% of energy	107 (24.2)
≥1% of energy	74 (16.7)
Dietary supplement use	
No	357 (80.6)
Yes	86 (19.4)
Physical activity (total metabolic equivalent h/d)	34.1 ± 3.5
Energy intake (kJ/d)	7314 ± 1741
Protein intake (% of energy)	13.8 ± 1.9
Total fat intake (% of energy)	29.5 ± 5.0
SFA intake (% of energy)	8.5 ± 2.0
MUFA intake (% of energy)	10.2 ± 2.1
PUFA intake (% of energy)	6.5 ± 1.3
Total n-3 PUFA intake (% of energy)	1.1 ± 0.3
EPA+DHA intake (% of energy)	0.2 ± 0.1
ALA intake (% of energy)	0.8 ± 0.2
Carbohydrate intake (% of energy)	55.1 ± 5.8
Total dietary fiber intake (g/4184 kJ)	7.1 ± 2.1
Magnesium intake (mg/4184 kJ)	125 ± 28
Vitamin C intake (mg/4184 kJ)	49 ± 23
Fruit intake (g/4184 kJ)	35.6 ± 33.8
Vegetable intake (g/4184 kJ)	120.5 ± 71.7
Fish and shellfish intake (g/4184 kJ)	29.6 ± 15.4
Dietary glycemic load (/4184 kJ)	79.6 ± 12.7

[a] Values are mean ± SD or number of subjects (%).

3. Results

Subject characteristics are shown in Table 1. Mean ± SD serum CRP concentration was 0.302 ± 0.727 mg/L, with a range of 0.025 to 7.100 mg/L. The prevalence of elevated

Table 2
Multivariate adjusted ORs and 95% CIs for elevated serum CRP concentrations (≥1.0 mg/L) by low or high intake of selected dietary variables in 443 Japanese women aged 18 to 22 years

	Category of dietary variable[a]	
	Low (n = 221)	High (n = 222)
Total fat intake (% of energy)[b]	26.1 (15.2-29.3)	32.4 (29.4-45.1)
n (%) with elevated CRP	13 (5.9)	12 (5.4)
Multivariate adjusted OR (95% CI)[c]	1.00	0.88 (0.38-2.02)
SFA intake (% of energy)[b]	7.1 (3.5-8.3)	9.7 (8.3-16.5)
n (%) with elevated CRP	11 (5.0)	14 (6.3)
Multivariate adjusted OR (95% CI)[c]	1.00	1.22 (0.52-2.83)
MUFA intake (% of energy)[b]	8.7 (4.4-10.1)	11.5 (10.1-17.1)
n (%) with elevated CRP	15 (6.8)	10 (4.5)
Multivariate adjusted OR (95% CI)[c]	1.00	0.62 (0.27-1.45)
PUFA intake (% of energy)[b]	5.5 (2.0-6.4)	7.3 (6.4-10.8)
n (%) with elevated CRP	14 (6.3)	11 (5.0)
Multivariate adjusted OR (95% CI)[c]	1.00	0.76 (0.33-1.76)
Total n-3 PUFA intake (% of energy)[b]	0.9 (0.3-1.1)	1.3 (1.1-2.6)
n (%) with elevated CRP	18 (8.1)	7 (3.2)
Multivariate adjusted OR (95% CI)[c]	1.00	0.33 (0.13-0.82)
EPA+DHA intake (% of energy)[b]	0.1 (0.02-0.2)	0.3 (0.2-1.3)
n (%) with elevated CRP	13 (5.9)	12 (5.4)
Multivariate adjusted OR (95% CI)[c]	1.00	0.81 (0.35-1.87)
ALA intake (% of energy)[b]	0.7 (0.2-0.8)	1.0 (0.8-1.6)
n (%) with elevated CRP	15 (6.8)	10 (4.5)
Multivariate adjusted OR (95% CI)[c]	1.00	0.62 (0.27-1.45)
Total dietary fiber intake (g/4184 kJ)[b]	5.8 (3.2-6.8)	8.0 (6.8-19.0)
n (%) with elevated CRP	13 (5.9)	12 (5.4)
Multivariate adjusted OR (95% CI)[c]	1.00	0.93 (0.41-2.12)
Magnesium intake (mg/4184 kJ)[b]	106 (64-121)	137 (121-277)
n (%) with elevated CRP	12 (5.4)	13 (5.9)
Multivariate adjusted OR (95% CI)[c]	1.00	0.99 (0.43-2.27)
Vitamin C intake (mg/4184 kJ)[b]	35 (7-45)	58 (45-178)
n (%) with elevated CRP	16 (7.2)	9 (4.1)
Multivariate adjusted OR (95% CI)[c]	1.00	0.49 (0.21-1.15)
Fruit intake (g/4184 kJ)[b]	13.4 (0-24.9)	47.4 (24.9-249.4)
n (%) with elevated CRP	13 (5.9)	12 (5.4)
Multivariate adjusted OR (95% CI)[c]	1.00	0.85 (0.37-1.93)
Vegetable intake (g/4184 kJ)[b]	71.3 (13.2-104.6)	149.5 (104.7-528.2)
n (%) with elevated CRP	14 (6.3)	11 (5.0)
Multivariate adjusted OR (95% CI)[c]	1.00	0.77 (0.33-1.77)
Fish and shellfish intake (g/4184 kJ)[b]	18.6 (1.7-27.2)	37.8 (27.2-100.5)
n (%) with elevated CRP	13 (5.9)	12 (5.4)
Multivariate adjusted OR (95% CI)[c]	1.00	0.83 (0.36-1.91)
Dietary glycemic load (/4184 kJ)[b]	71.4 (35.2-79.0)	87.7 (79.0-121.3)
n (%) with elevated CRP	12 (5.4)	13 (5.9)
Multivariate adjusted OR (95% CI)[c]	1.00	1.16 (0.50-2.71)

[a] Subjects were divided into 2 categories according to the median value of dietary intake.
[b] Values are median (range).
[c] Adjusted for residential block (north [Kanto and Tohoku], central [Tokai and Hokuriku], and south [Kyushu and Chugoku]), size of residential area (city with a population of ≥1 million, city with a population of <1 million, and town and village), current smoking (yes or no), alcohol drinking (nondrinker, >0% to <1% of energy, or ≥1% of energy), dietary supplement use (yes or no), physical activity (total metabolic equivalent hours per day, continuous), and BMI (kg/m^2, continuous).

CRP concentration was 5.6%. Overall, subjects were characterized by a relatively low BMI (mean ± SD, 21.3 ± 2.9 kg/m^2) and a low smoking rate (3.4%). About three fifths (59.1%) of subjects were alcohol nondrinkers, and dietary supplements were used by about one fifth (19.4%) of subjects. Mean ± SD dietary intake was 1.1 ± 0.3% of energy for total n-3 PUFA, 0.2 ± 0.1% of energy for EPA + DHA, 0.8 ± 0.2% of energy for ALA, and 49 ± 23 mg/1000 kcal for vitamin C.

Table 2 shows associations between dietary intake and elevated CRP concentrations. After adjustment for possible confounders, total n-3 PUFA intake was significantly inversely associated with serum CRP concentration. The multivariate adjusted ORs of elevated CRP concentrations for women with an intake below and above the median value (1.1% of energy) were 1.00 and 0.33 (95% CI, 0.13-0.82; $P = .02$), respectively. Intake of EPA + DHA and ALA was not associated with elevated CRP concentrations ($P = .62$ and $P = .27$, respectively). Vitamin C intake was independently inversely associated with elevated CRP concentrations, although the association was nonsignificant ($P = .10$). No clear associations were observed for other dietary factors examined including total fat, SFA, MUFA, PUFA, total dietary fiber (as well as soluble and insoluble dietary fiber; data not shown), magnesium, fruits, vegetables, fish and shellfish, or dietary glycemic load ($P = .27$ to $P = .99$).

4. Discussion

In this study of young Japanese women, we found that a higher intake of total n-3 PUFA was associated with a lower prevalence of elevated CRP concentrations, independently of potential confounding factors, including BMI. Similar inverse relations have been observed not only in middle-aged American women [6] but also in elderly Japanese men and women [8]. The inverse relation between total n-3 PUFA intake and circulating CRP is biologically reasonable, given that n-3 PUFA possesses anti-inflammatory properties, such as the ability to inhibit inflammatory cytokine production [31]. Conversely, we found no significant association between

intake of EPA + DHA and ALA (main components of n-3 PUFA) and elevated CRP. Previous studies of this association have shown inconsistent findings: a significant inverse association was seen with intake of EPA + DHA but not ALA in elderly Japanese men and women [8] and with ALA but not EPA + DHA in middle-aged American women [6], whereas no association with either EPA + DHA or ALA intake was seen in middle-aged American men and women [5]. Further investigation of the association between subtypes of n-3 PUFA and CRP would be of interest.

Given that our subjects were selected female dietetic students rather than a random sample of Japanese women, the present results may not be extrapolative to the general Japanese population. In addition, because the study population consisted of generally healthy persons, the clinical relevance of our findings remains to be elucidated. In this regard, the prevalence of elevated serum CRP concentrations (5.8%) was quite low compared with that in young Finnish adults (33.2%) [32]. It is possible that the absence of associations of CRP concentration with intake of nutrients (other than total n-3 PUFA) and foods and dietary glycemic load was due to the low base rate of elevated CRP concentration in our sample. Nevertheless, our results should provide valuable insight from a prevention perspective. The relatively healthy dietary habits and low prevalence of elevated CRP levels in this population of young healthy and lean women suggests that greater differences might be seen in other populations.

Although several studies have shown a significant inverse relation of vitamin C intake with circulating CRP levels [13,14], the inverse association seen here did not reach statistical significance. In addition, although previous studies have shown variously favorable effects on circulating CRP levels of dietary fiber [4,9,10], magnesium [11,12], EPA + DHA [8], ALA [6], fruits [14-16], vegetables [14-16], and fish [17] and adverse effects of SFA [5,7] and dietary glycemic load [18], we saw none of these associations here. These discrepancies may be at least partly explained by the different populations investigated, the different dietary assessment methods used, and the differences in the number and type of variables used as confounding factors.

Several limitations of our study warrant mention. First, we used a self-administered semiquantitative dietary assessment questionnaire for dietary data collection [23-25]. Although the questionnaire had been previously validated, actual dietary habits were not observed, so the results should be interpreted cautiously. To minimize the influence of dietary misreporting, an ongoing controversy in studies that collect dietary information using self-report instruments, we used energy-adjusted values of dietary intake [33]. Second, a single measurement of CRP may not be reliable, introducing random errors [34]. However, although this kind of error would tend to result in bias toward attenuating the relation, we did find a significant relation between total n-3 PUFA intake and elevated serum CRP concentrations. Third, because our sample size was relatively small, statistical power may have been insufficient to allow the detection of associations between several dietary factors and circulating CRP levels. Fourth, we were unable to include nutrient intake from dietary supplements in the analysis because of the lack of a reliable composition table of dietary supplements in Japan. However, the exclusion of dietary supplement users from analysis did not materially alter the results (data not shown), suggesting it unlikely that dietary supplements had a major effect on the findings. Fifth, although we attempted to adjust for a wide range of potential confounding variables, we are unable to rule out residual confounding. In addition, physical activity was assessed relatively roughly from only 5 activities, which might not have been sufficient. Moreover, although oral contraceptive affects circulating CRP [32], we unfortunately did not assess oral contraceptive use in the present study. Further research examining association of lifestyle factors with circulating CRP in women of reproductive age should take into account oral contraceptive use. Finally, the cross-sectional nature of the study does not permit the assessment of causality owing to the uncertain temporality of the association.

To conclude, after adjustment for potential confounding factors, including BMI, we observed an inverse relation between total n-3 PUFA intake and elevated serum CRP concentrations in this cross-sectional study in young Japanese women. This finding is consistent with previous cross-sectional findings in middle-aged American women [6] and elderly Japanese men and women [8]. Dietary modification to increase intake of total n-3 PUFA even in earlier stage of life may thus be an important strategy from the standpoint of prevention. Further prospective studies or intervention trials should be undertaken to confirm the relation between total n-3 PUFA intake and circulating CRP concentration.

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