Hardness (difficulty of chewing) of the habitual diet in relation to body mass index and waist circumference in free-living Japanese women aged 18–22 y[1–3]

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ABSTRACT

Background: Animal studies suggest the beneficial effect of hardness of diet on body weight and adiposity. No human studies have examined hardness of diet in relation to obesity.

Objective: We examined cross-sectional associations of hardness of the habitual diet with body mass index (BMI; in kg/m^2) and waist circumference in free-living humans.

Design: Subjects were 454 female Japanese dietetic students aged 18–22 y. Dietary hardness was assessed as an estimate of masticatory muscle activity for the habitual diet (ie, the difficulty of chewing the food). The consumption of a total of 107 foods was estimated by means of a self-administered, comprehensive diet history questionnaire, and masticatory muscle activity during the ingestion of these foods was estimated according to published equations. Waist circumference was measured at the level of the umbilicus.

Results: Mean BMI was 21.4 (95% CI: 21.1, 21.6), and mean waist circumference was 73.6 (72.9, 74.3) cm. Mean dietary hardness was 178 (175, 181) mV · s/1000 kcal. Dietary hardness was not significantly associated with BMI. However, it was negatively associated with waist circumference (P for trend = 0.005). This association remained after adjustment not only for potential confounding factors (P for trend = 0.028) but also for BMI (P for trend = 0.002).

Conclusions: Whereas no association between dietary hardness and BMI was seen, increasing dietary hardness was associated with lower waist circumference even after adjustment for BMI in free-living young Japanese women. This finding could make innovative contributions to the literature and raise issues for future studies regarding diet and obesity. *Am J Clin Nutr* 2007;86:206–13.

KEY WORDS Hardness of diet, body mass index, waist circumference, Japanese, women, diet history questionnaire, epidemiology

INTRODUCTION

Because the human genome has hardly changed since the emergence of behaviorally modern humans ≈10 000 y ago, contemporary humans are still genetically adapted for the foods consumed by our remote ancestors (1–5). The dietary choices of that time would necessarily have been limited to minimally processed or unprocessed—and, often, uncooked—wild plant and animal foods (2). In contrast, the contemporary diet in affluent societies mainly consists of foods that could not have been regularly consumed before the development of agriculture, industrialization, and advanced technology such as food-processing procedures; these foods include dairy products, cereals, refined cereals, refined sugars, refined vegetable oils, fatty meats, salt, and combinations of these foods (3). The collision of our ancient genome with the new conditions of life in affluent nations, including the dietary qualities of recently introduced foods is considered to be the ultimate factor underlying diseases of civilization, including obesity (3–5). Given that probability, the differences between the ancient dietary patterns and those currently prevalent in industrialized countries appear to have important implications for the prevention and treatment of contemporary chronic diseases, including obesity. A dietary characteristic that would differ greatly between the ancient dietary patterns and the contemporary dietary patterns in developed societies is hardness of the diet, referred to hereafter as dietary hardness.

However, no human studies have examined with diligence the possible association between dietary hardness and diseases of civilization, such as obesity. In contrast, several studies in mice

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(6, 7) and rats (8, 9) suggested the beneficial effect of dietary hardness on obesity. In this preliminary study, we tried to examine hardness of the habitual diet in relation to body mass index (BMI; in kg/m^2) and waist circumference (WC; in cm) among young free-living Japanese women. For this examination, we assessed dietary hardness by using an estimate of masticatory muscle activity for the habitual diet, obtained with data on the consumption of a total of 107 foods estimated by a self-administered comprehensive diet history questionnaire (DHQ) (10–12) and data on masticatory muscle activities during the ingestion of these foods estimated according to published equations (13).

SUBJECTS AND METHODS

Subjects

The present study was based on a multicenter nutritional survey conducted in February and March 2006 among female dietetics students from 10 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. Subjects who responded positively were then provided detailed written and oral explanations of the general purpose and procedure of the survey. A total of 474 women took part. For the present analysis, we selected 454 women who met the following 3 inclusion criteria: they were 18–22 y old (n = 467); were not currently receiving dietary counseling from a doctor or dietitian (n = 468); and had a reported energy intake in the range of 1000–3500 kcal/d (n = 467).

Written informed consent was obtained from each subject, and also from a parent for subjects aged <20 y. The study protocol was approved by the Ethics Committee of the Japanese National Institute of Health and Nutrition (of Japan).

Dietary assessment

Dietary habits during the preceding month were assessed by using a self-administered comprehensive DHQ (10–12). Responses to the DHQ, as well as those to a lifestyle questionnaire, were checked at least twice for completeness. 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 7 sections: general dietary behavior; major cooking methods; consumption frequency and amount of 6 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 (≥1 time/wk) 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 books of recipes for Japanese dishes (10). Estimates of dietary intake for a total of 150 food and beverage items (including 5 seasonings), energy, and nutrients were calculated by using an ad hoc computer algorithm for the DHQ 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). Detailed descriptions of the methods used to calculate dietary intake and the validity of the DHQ with respect to nutrients have been published elsewhere (10–12). Pearson's correlation coefficients between the DHQ and 3-d estimated dietary records were 0.48 for energy and 0.48–0.55 for macronutrients in 47 women (10). In addition, Pearson's correlation coefficient between the DHQ and 16-d weighed dietary records was 0.71 for dietary fiber in 92 women, and the mean value of Spearman's correlation coefficients of food groups was 0.44 (range: 0.13–0.77; S Sasaki, unpublished observations, 2006).

Estimation of dietary hardness

In the present study, dietary hardness was assessed as estimated masticatory muscle activity needed for the habitual diet (ie, the difficulty of chewing the food in the diet). Whereas the habitual diet was assessed by DHQ (10–12) as described above, estimates of masticatory muscle activity for each food in the DHQ were obtained from equations published by Yanagisawa et al (13). Those authors measured the activities of 6 muscle regions (mV · s) involved in mastication (right and left masseters and anterior and posterior temporalis) by using electromyography during the ingestion of the same volume (1.3 × 1.3 × 1.3 cm) of 16 selected foods with various physical properties by 20 healthy Japanese adults (10 men and 10 women) with a mean age of 21 y. They found that masticatory muscle activities (mV · s/2.197 cm^3) were highly correlated with the physical properties of foods (ie, firmness, cohesiveness, and strain) as measured with a texturometer (GTX-2; Zenken KK Inc, Chiba, Japan) and developed the following equations (13):

$$\text{Masticatory muscle activity} = 0.6586 \times \ln(\text{firmness} \times \text{cohesiveness} \times \text{strain} \times 10) - 0.0307 \quad (1)$$

where $R^2 = 0.89$;

$$\text{Masticatory muscle activity} = 0.2718 \times \text{firmness} + 0.0335 \times \text{strain} - 0.0030 \quad (2)$$

where $R^2 = 0.89$; or

$$\text{Masticatory muscle activity} = 0.3081 \times \text{firmness} + 0.3300 \quad (3)$$

where $R^2 = 0.81$.

Using the information on the physical properties of foods they had measured earlier with a texturometer (15), Yanagisawa et al (13) then estimated masticatory muscle activities for a total of 144 foods according to one of their equations, by using the available variables (ie, firmness, cohesiveness, and strain).

They did not, however, cross-validate the equations to show their applicability (13). We therefore conducted a cross-evaluation by using data reported by Shiono et al (16). Those authors measured the activities of 4 muscle regions (mV · s) involved in mastication (right and left masseters and anterior temporalis, but not posterior temporalis) by using electromyography during the ingestion of standard-sized bites (2.4–44.5 g) of 46 selected foods with various physical properties by 6 healthy Japanese adults (3 men and 3 women) aged 23–27 y. By careful direct matching, information on masticatory muscle activities

for a total of 18 foods was available from Shiono et al (16) and information on physical properties was available from Yanagisawa et al (15). Pearson's correlation coefficient between masticatory muscle activities measured by Shiono et al (mV · s/g food) (16) and those estimated by using physical property values as described by Yanagisawa et al [mV · s/g food (= mV · s/2.197 cm^3 divided by 2.197, assuming that the density of all foods = 1)] (13) was 0.88 among these 18 foods. This high correlation suggests the applicability of the equations developed by Yanagisawa et al, despite the differences in masticatory muscles measured and in the amounts of foods consumed in the studies of Yanagisawa et al (13) and Shiono et al (16).

We directly matched each food item on the DHQ (n = 150) (10–12) with foods for which information on masticatory muscle activities was available (n = 144) from Yanagisawa et al (13). During the calculation of dietary hardness, we excluded from the 150 food items on the DHQ beverages (22 items), soups (4 items), seasonings including fat and oil (16 items), and water (1 item). Foods for which masticatory muscle activities had not been determined (21 items) were assigned a value according to that of a comparable food. Because the physical properties (and hence the hardness, or difficulty of chewing) of vegetables are greatly influenced by cooking with heat (13), we took those influences into account as much as possible. For tomatoes and cucumbers, we used values for raw tomatoes and raw cucumbers, respectively, because these vegetables are usually consumed without heating in Japan. For cabbage, we used a weighted mean of a value for raw cabbage and that for boiled leafy vegetables (because of a lack of information on boiled cabbage), based on the ratio of the observed consumption (g/d) of raw cabbage to that of cabbage cooked with heat (ie, 4:6) in 92 women (S Sasaki, unpublished observations, 2006). For carrots, we used a weighted mean of a value for raw carrots and that for boiled carrots, based on the ratio of the observed consumption (g/d) of raw carrots to that of carrots cooked with heat (ie, 3:7) in 92 women (S Sasaki, unpublished observations, 2006). For other vegetables, we used values adjusted for cooking with heat, given that these foods are usually consumed after cooking with heat in Japan. Dietary hardness was calculated as the sum of the products of estimated masticatory activities (mV · s/2.197 cm^3) and the volume of food consumed (cm^3/d) divided by 2.197. For the estimation of food volume, we simply converted weight in grams to weight in cubic centimeters for all of the foods, on the assumption that the density of all foods = 1. Because the crude value of dietary hardness was strongly correlated with energy intake (Pearson's correlation coefficient = 0.75), the energy-adjusted value (mV · s/1000 kcal) was used in the present study. Estimates of masticatory muscle activity for the 107 food items used to calculate dietary hardness are presented in **Table 1**. We could not investigate the validity of the DHQ against the 16-d dietary records (which we used to investigate the validity of other dietary variables, as described above) in assessing dietary hardness, because an insufficient number of foods (n = 144 items) with information on hardness (ie, masticatory muscle activity) (13) prevented the calculation of dietary hardness by the 16-d dietary records.

Anthropometric measurements

Body height was measured to the nearest 0.1 cm while the subjects were standing and not wearing shoes. Body weight was measured to the nearest 0.1 kg while the subjects were wearing lightweight indoor clothing. WC was measured to the nearest 0.1 cm at the level of the umbilicus. The measurement was taken at the end of a normal respiration while the subjects were standing erect and with the arms at the side and the feet together.

Other variables

In the lifestyle questionnaire, the subject reported her residential area, which was grouped into 1 of 3 regions: northern (Kanto and Tohoku), central (Tokai, Hokuriku, and Kinki), or southern (Kyushu and Chugoku) Japan. The residential areas were also grouped into 3 categories according to population size (city with population ≥ 1 million, city with population < 1 million, or town or village). Current smoking status (yes or no) and whether the subject was currently trying to lose weight (yes or no) were self-reported in the lifestyle questionnaire. Physical activity was computed as the average metabolic equivalent-hours [MET · h/d (17)], 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. Rate of eating (slow, medium, or fast) was self-reported as part of the DHQ.

Statistical analysis

All statistical analyses were performed with SAS software (version 8.2; SAS Institute Inc, Cary, NC). With the use of the PROC GLM procedure, linear regression models were constructed to examine the association of dietary hardness with BMI and WC. For analyses, subjects were categorized into quintiles according to dietary hardness values (mV · s/1000 kcal). Mean (± SE) values of BMI and WC were calculated by quintiles of dietary hardness with or without adjustment for potential confounding factors, including residential area, size of residential area, current smoking, currently trying to lose weight, physical activity (total MET · h/d, continuous), rate of eating, and energy intake (kcal/d, continuous). In the analysis of WC, BMI (continuous) was also included as a confounding variable. We also conducted analyses with further adjustment for nutrient intakes, including protein (% of energy, continuous), fat (% of energy, continuous), and dietary fiber (g/1000 kcal, continuous). Because alcohol intake was extremely low ($\bar{x}$: 1.4 g/d), alcohol intake was not considered a confounding factor. We tested for linear trends with increasing levels of dietary hardness by assigning each participant the median value for the category and modeling this value as a continuous variable. All reported P values are 2-tailed, and $P < 0.05$ was considered significant.

RESULTS

Basic characteristics of the subjects are shown in **Table 2**. Mean BMI was 21.4 (95% CI: 21.1, 21.6), and mean WC was 73.6 (72.9, 74.3) cm. Mean dietary hardness was 178 (175, 181) mV · s/1000 kcal (range: 101–289 mV · s/1000 kcal). The top contributor to dietary hardness was well-milled rice (27.0%), and next were spaghetti (4.1%), pork (3.9%), green leafy vegetables (3.7%), and cabbage (3.4%), as shown in Table 1. Potential confounding factors are shown by quintile of dietary hardness in **Table 3**. There was a negative association between dietary hardness and rate of eating. Dietary hardness was negatively associated with energy and fat intakes and positively associated with protein and dietary fiber intakes.

TABLE 1
Hardness of the 107 food items used in the present study[1]

Food item	Hardness	Contribution to dietary hardness[2]
	mV · s/1000 kcal	*%*
Well-milled rice	225	27.04 ± 15.22[3]
Spaghetti	339	4.07 ± 3.78
Pork	236	3.87 ± 2.97
Green leafy vegetables	1701	3.65 ± 3.32
Cabbage	2546	3.44 ± 3.04
Chicken	351	2.85 ± 2.37
Eggs	135	2.41 ± 1.50
Beef	345	2.37 ± 2.39
Japanese noodles (buckwheat and Japanese wheat noodles)	278	2.37 ± 2.19
Chinese cabbage	2404	1.84 ± 2.10
Mushrooms	2560	1.80 ± 1.66
Instant noodles	236	1.74 ± 2.48
White bread	76	1.60 ± 1.60
Apples	664	1.49 ± 2.19
Carrots	857	1.45 ± 1.07
Brown rice	230	1.41 ± 5.91
Well-milled rice with germ	227	1.33 ± 6.26
Wakame seaweed	3265	1.32 ± 1.50
Salted pickles (excluding plums)	2652	1.21 ± 2.15
Chinese noodles	236	1.19 ± 2.13
Japanese bread with a sweet filling	70	1.16 ± 1.46
Ground beef and pork	198	1.12 ± 0.98
Broccoli[4]	1360	1.11 ± 1.38
Pizza	209	1.11 ± 1.97
Lettuce	3971	1.05 ± 1.21
Onions	555	0.97 ± 0.75
Natto (fermented soybeans)	129	0.94 ± 1.11
Oily fish	143	0.92 ± 0.82
Cucumbers	3327	0.82 ± 1.15
Shrimp	711	0.79 ± 0.68
Squid and octopus	691	0.79 ± 0.87
Well-milled rice mixed with barley	259	0.77 ± 4.91
White meat fish	229	0.76 ± 0.65
Bean sprouts	2892	0.74 ± 0.86
Oranges	212	0.74 ± 1.18
Red meat fish	225	0.73 ± 0.65
Dried fish	265	0.70 ± 1.12
Green peppers	2417	0.65 ± 0.91
70%-Milled rice	225	0.63 ± 4.64
Cornflakes[4]	170	0.60 ± 1.66
Tofu (soybean curd) products	266	0.59 ± 0.98
French fries	218	0.59 ± 0.80
Cheese	91	0.58 ± 0.90
Sweet potatoes, yams, and taro	175	0.52 ± 0.55
Japanese-style pancakes[4]	112	0.50 ± 0.73
Small fish with bones	766	0.47 ± 0.66
Butter roll	80	0.46 ± 1.00
Bananas	165	0.45 ± 0.80
Ground fish meat products	327	0.42 ± 0.64
Cakes	50	0.41 ± 0.46
Burdock	578	0.40 ± 0.61
Croissant	46	0.38 ± 0.76
Tomatoes	568	0.38 ± 0.42
Potatoes	101	0.37 ± 0.38
Rice crackers	112	0.36 ± 0.56
Ham and sausages	97	0.36 ± 0.39
Radishes	450	0.35 ± 0.30
Sweetened yogurt[4]	78	0.32 ± 0.58
Konnyaku (devil's tongue jelly)	6309	0.32 ± 0.53
Japanese sweets with azuki beans	138	0.31 ± 0.43
Half-milled rice	227	0.30 ± 2.70
Tofu	74	0.30 ± 0.26
Shellfish other than oysters	1042	0.29 ± 0.41
Doughnuts	51	0.28 ± 0.46
Cookies and biscuits	36	0.26 ± 0.36
Eggplants	1337	0.26 ± 0.39
Strawberries	317	0.24 ± 0.28
Bacon	86	0.24 ± 0.34
Pancakes[4]	72	0.23 ± 0.57
Canned tuna	101	0.22 ± 0.31
Snacks made from wheat flour	57	0.21 ± 0.33
Boiled beans	75	0.21 ± 0.35
Liver	468	0.19 ± 0.40
Nonsweetened yogurt[4]	85	0.17 ± 0.54
Nutritional supplement bars[4]	131	0.16 ± 0.61
Chocolates[4]	9	0.16 ± 0.22
Moderately sweetened yogurt[4]	81	0.15 ± 0.46
Salted pickled plums[4]	626	0.15 ± 0.25
Raisins	199	0.14 ± 0.38
Oysters	885	0.14 ± 0.25
Ice cream (unspecified varieties)[4]	24	0.13 ± 0.20
Pumpkins	75	0.13 ± 0.15
Japanese sweets without azuki beans	145	0.12 ± 0.22
Ice cream (regular)[4]	23	0.11 ± 0.31
Nuts (not peanuts)	95	0.11 ± 0.18
Cauliflower[4]	1413	0.11 ± 0.45
Peanuts	78	0.11 ± 0.29
Boiled fish, shellfish, and seaweed in soy sauce	246	0.10 ± 0.27
Potato chips	42	0.09 ± 0.15
Jellies	73	0.08 ± 0.14
Kiwi fruit[4]	171	0.07 ± 0.18
Candies, caramels, and chewing gum[4]	13	0.07 ± 0.11
Lotus root[4]	183	0.07 ± 0.15
Canned fruits	146	0.05 ± 0.09
Persimmons	504	0.04 ± 0.18
Cottage cheese	293	0.04 ± 0.12
Pears	704	0.03 ± 0.23
Eel	41	0.02 ± 0.05
Laver (dried, edible seaweed)[4]	196	0.02 ± 0.02
Fish eggs[4]	25	0.02 ± 0.03
Salted fish intestines	467	0.02 ± 0.05
Jam and marmalade[4]	10	0.02 ± 0.04
Ice cream (premium)[4]	25	0.01 ± 0.10
Peaches	279	0.01 ± 0.07
Melons	126	0.00 ± 0.02
Grapes[4]	39	0.00 ± 0.01
Watermelons	201	0.00 ± 0.02

[1] These 107 food items from the 150 items in the diet history questionnaire were used for the calculation of dietary hardness. The remaining 43 items not used consisted of 22 beverages [fruit juice (100%), other fruit juice, tomato juice, vegetable juice, beer, sake, shochu, shochu mixed with water or a carbonated beverage, whiskey, wine, green and oolong tea, black tea, coffee, cocoa, lactic acid bacteria beverages, sugar-sweetened soft drinks, sugar-free soft drinks, nutritional supplement drinks, full-fat milk, low-fat milk, skim milk, and cream or creamer added to coffee], 4 soups (corn soup, Chinese soup, soup consumed with noodles, and water for miso soup), 16 seasonings including fat and oil (sugar for coffee and black tea, sugar used during cooking, butter, margarine, mayonnaise, salad dressing, fat-free salad dressing, oil used during cooking, miso as seasoning, miso for miso soup, ketchup, table salt, salt used during cooking, soy sauce, curry and roux in stew, and artificial sweeteners), and drinking water. Food items are listed in the descending order of their mean contribution to overall dietary hardness.

[2] Based on the data for subjects in the present study (454 Japanese women aged 18–22 y).

[3] $\bar{x}$ ± SD (all such values).

[4] These 21 food items were assigned the hardness value of a comparable food.

TABLE 2
Basic characteristics of 454 Japanese women aged 18–22 y

	Value
Age (y)	19.6 ± 1.0[1]
Body height (cm)	158.1 ± 5.5
Body weight (kg)	53.4 ± 8.1
BMI (kg/m^2)	21.4 ± 3.0
Waist circumference (cm)	73.6 ± 7.4
Area of residence [*n* (%)]	
North (Kanto and Tohoku)	267 (59)
Central (Tokai, Hokuriku, and Kinki)	85 (19)
South (Kyushu and Chugoku)	102 (22)
Size of residential area [*n* (%)]	
City with a population ≥1 million	80 (18)
City with a population <1 million	334 (74)
Town or village	40 (9)
Current smoking [*n* (%)]	
No	441 (97)
Yes	13 (3)
Currently trying to lose weight [*n* (%)]	
No	342 (75)
Yes	112 (25)
Physical activity (total metabolic equivalents · h/d)	34.1 ± 3.5
Rate of eating [*n* (%)]	
Slow	140 (31)
Medium	144 (32)
Fast	170 (37)
Energy intake (kcal/d)	1761 ± 406
Protein intake (% of energy)	13.9 ± 1.9
Fat intake (% of energy)	29.5 ± 5.0
Carbohydrate intake (% of energy)	55.1 ± 5.8
Dietary fiber intake (g/1000 kcal)	7.1 ± 2.1
Dietary hardness (mV · s/1000 kcal)	178 ± 31

[1] $\bar{x}$ ± SD (all such values).

Mean values of BMI and WC across quintiles of dietary hardness are shown in **Table 4**. Dietary hardness was not significantly associated with BMI, regardless of adjustment for potential confounding factors. Conversely, dietary hardness was significantly and negatively associated with WC (in model 1, the mean difference in WC between the lowest and highest quintiles of dietary hardness was −2.9 cm; P for trend = 0.005). The significant negative association between dietary hardness and WC remained after adjustment for potential confounding factors (in model 2, mean difference: −2.7 cm; P for trend = 0.028) and also BMI (in model 4, mean difference: −2.4 cm; P for trend = 0.002). This inverse association seemed mainly due to the composition of the diet, because it disappeared after further adjustment for dietary intake (models 3 and 5).

DISCUSSION

To our knowledge, this is the first study to examine dietary hardness in relation to BMI and WC in humans. We found that, whereas there was no association with BMI, dietary hardness was negatively associated with WC even after adjustment for BMI in free-living young Japanese women. No human studies have examined the association between dietary hardness and obesity, but several animal studies have suggested the beneficial effect of a hard diet on obesity. Mice fed a hard diet from age 4 wk had a significantly lower body weight at age 36 wk than did mice fed a normal diet (6). In addition, body-weight gain from 4 to 9 wk of age was significantly smaller in male (but not female) mice fed a hard diet than in those fed a soft diet (7), and body-weight gain at age 6 wk was significantly smaller in rats fed a hard diet from age 1 wk than in those fed a soft diet (8). Furthermore, rats fed a hard diet from age 4–26 wk had significantly lower body weight and abdominal white adipose tissue than did those fed a soft diet (9).

TABLE 3
Selected characteristics of 454 Japanese women aged 18–22 y according to quintile (Q) of dietary hardness

	Q1 (n = 90)	Q2 (n = 91)	Q3 (n = 91)	Q4 (n = 91)	Q5 (n = 91)	P[1]
Dietary hardness (mV · s/1000 kcal)	137 ± 13[2]	161 ± 5	176 ± 5	193 ± 6	223 ± 19	
Area of residence (%)						< 0.0001
North (Kanto and Tohoku)	72	68	57	54	43	
Central (Tokai, Hokuriku, and Kinki)	17	20	14	15	27	
South (Kyushu and Chugoku)	11	12	29	31	30	
Size of residential area (%)						0.10
City with a population ≥1 million	18	16	19	24	11	
City with a population <1 million	79	78	68	66	77	
Town or village	3	5	13	10	12	
Current smokers (%)	1	7	3	0	3	0.68
Subjects currently trying to lose weight (%)	26	29	21	29	20	0.42
Physical activity (total metabolic equivalents · h/d)	34.7 ± 5.2	33.8 ± 2.7	33.6 ± 2.4	34.1 ± 3.0	34.1 ± 3.3	0.51
Rate of eating (%)						0.03
Slow	32	23	27	37	34	
Medium	27	31	32	32	37	
Fast	41	46	41	31	29	
Energy intake (kcal/d)	1885 ± 419	1770 ± 410	1782 ± 375	1665 ± 394	1704 ± 403	0.0006
Protein intake (% of energy)	13.1 ± 1.6	13.4 ± 1.7	13.8 ± 1.6	14.1 ± 1.9	15.0 ± 1.9	< 0.0001
Fat intake (% of energy)	31.0 ± 4.5	30.3 ± 5.6	29.8 ± 4.9	27.7 ± 5.0	28.6 ± 4.6	< 0.0001
Dietary fiber intake (g/1000 kcal)	6.0 ± 1.2	6.3 ± 1.2	6.6 ± 1.5	7.1 ± 1.8	9.5 ± 2.4	< 0.0001

[1] For continuous variables, a linear trend test was used with the median value in each quintile as a continuous variable in linear regression; a Mantel-Haenszel chi-square test was used for categorical variables.
[2] $\bar{x}$ ± SD (all such values).

TABLE 4
BMI and waist circumference according to quintile (Q) of dietary hardness in 454 Japanese women aged 18–22 y

	Q1 (n = 90)	Q2 (n = 91)	Q3 (n = 91)	Q4 (n = 91)	Q5 (n = 91)	P for trend[1]
Dietary hardness (mV · s/1000 kcal)	142 (101–152)[2]	163 (153–167)	176 (168–183)	192 (184–204)	216 (205–289)	
BMI (kg/m^2)						
Model 1[3]	21.4 ± 0.3[4]	21.6 ± 0.3	21.3 ± 0.3	21.4 ± 0.3	21.1 ± 0.3	0.47
Model 2[5]	21.3 ± 0.3	21.5 ± 0.3	21.3 ± 0.3	21.4 ± 0.3	21.2 ± 0.3	0.73
Model 3[6]	21.1 ± 0.3	21.4 ± 0.3	21.2 ± 0.3	21.3 ± 0.3	21.7 ± 0.4	0.38
Waist circumference (cm)						
Model 1	75.0 ± 0.8	74.4 ± 0.8	73.0 ± 0.8	73.8 ± 0.8	71.9 ± 0.8	0.005
Model 2	74.9 ± 0.8	74.0 ± 0.8	73.1 ± 0.8	73.9 ± 0.8	72.2 ± 0.8	0.028
Model 3	74.3 ± 0.8	73.7 ± 0.8	72.9 ± 0.8	73.7 ± 0.8	73.6 ± 0.9	0.63
Model 4[7]	74.9 ± 0.5	73.7 ± 0.5	73.1 ± 0.5	73.8 ± 0.5	72.5 ± 0.5	0.002
Model 5[8]	74.8 ± 0.5	73.6 ± 0.5	73.1 ± 0.5	73.8 ± 0.5	72.9 ± 0.6	0.063

[1] A linear trend test was used with the median value in each quintile as a continuous variable in linear regression.
[2] Median; range in parentheses (all such values).
[3] Crude model.
[4] $\bar{x}$ ± SE (all such values).
[5] Adjusted for residential area (Kanto and Tohoku in the north; Tokai, Hokuriku, and Kinki in the central area; or Kyushu and Chugoku in the south), size of residential area (city with a population ≥1 million, city with a population <1 million, or town or village), current smoking (yes or no), currently trying to lose weight (yes or no), physical activity (total metabolic equivalents · h/d, continuous), rate of eating (slow, medium, or fast), and energy intake (kcal/d, continuous).
[6] Adjusted for variables used in model 2, protein and fat intake (% of energy, continuous), and dietary fiber intake (g/1000 kcal, continuous).
[7] Adjusted for variables used in model 2 and BMI (in kg/m^2, continuous).
[8] Adjusted for variables used in model 3 and BMI (in kg/m^2, continuous).

We do not know why we found unexpected null association with BMI (but found the expected inverse association with WC). Several limitations of the present study, such as the narrow range of BMIs in the subjects, the study's cross-sectional design, and the use of a new and as yet unestablished method for assessing dietary hardness, may at least partly explain the null finding on BMI. Alternatively, the difference in abdominal white adipose tissue was much larger (22%) than that in body weight (6%) between rats fed a soft and those fed a hard diet (9), which may suggest that dietary hardness affects abdominal obesity (eg, WC) more strongly than it affects overall obesity (eg, BMI).

The negative association between dietary hardness and WC was independent of energy intake. Less body weight gain with a hard diet was related to decreased food intake in a study of rats (8). Conversely, the effect of dietary hardness on obesity was independent of the amount of foods consumed in other studies of mice (6) and rats (9), which may be due to increased thermogenesis (9) or unknown mechanisms. However, the negative association between dietary hardness and WC was not independent of diet composition, because that association disappeared after control for dietary composition. This finding is not consistent with findings from animal studies, because dietary hardness had a beneficial effect on obesity independent of diet composition (6–9). However, the question of whether the association of dietary hardness with obesity is independent of dietary composition should be examined and interpreted with caution, because, whereas dietary hardness can freely be changed in animal models while dietary composition remains constant, dietary hardness is associated with dietary composition in the diet of free-living humans. In the present study, greater dietary hardness was associated with healthier dietary patterns, including lower energy and fat and higher protein and dietary fiber. Several human studies have supported the favorable effects of healthy dietary patterns, including a high intake of dietary fiber (18–21) and a low intake of dietary fat (18, 19), on WC, which does not conflict with our finding.

Several limitations of the present study should be acknowledged. First, our subjects were selected female dietetics students, not a random sample of Japanese women, and the exact response rate was unknown because of our recruitment procedure; these elements of the design may produce recruitment bias. Thus, it may be that our results cannot be extrapolated to the general Japanese population.

Second, because this was a cross-sectional study, reverse causation may have occurred. However, it is unlikely that subjects with a large WC would intentionally change the hardness of their diet as a result of an increase in WC, because the notion that dietary hardness is associated with a measure of obesity is not well known. Furthermore, adjustment for intentional dietary change within the preceding year (yes or no), assessed as part of the DHQ, did not materially change the present results (data not shown). It is therefore reasonable to consider that our findings are not due to reverse causation.

Third, our DHQ was not designed specifically to measure dietary hardness, and the validity of the DHQ with respect to dietary hardness was unknown. The satisfactory validity of the DHQ for a wide range of nutrients and foods (10–12; S Sasaki, unpublished observations, 2006), however, may provide some reassurance. In addition, the DHQ may not adjust sufficiently for cooking methods in the calculation of dietary hardness. Our mean estimate of dietary hardness [crude $\bar{x}$ (±SD): 312 ± 82 mV · s/d; range: 140–647 mV · s/d] was higher than that assessed by 3-d dietary records in a group of 140 women aged 18–23 y (267 ± 69 mV · s/d; 109–523 mV · s/d) (22), although the estimation of dietary hardness by using dietary records would be less reliable because the database of hardness (ie, masticatory muscle activity) is limited to a few food items (13). Moreover, we simply converted weight in grams to weight in

cubic centimeters for all foods, assuming that the density of all foods = 1, even though for some foods that are high in air content (eg, snack foods), weight and volume are not directly proportional (23). Nevertheless, foods making the greatest contribution to dietary hardness in the present study did not seem to have this disproportional relation between weight and volume (*see* Table 1). Because the procedure we used provides only an approximation of the actual hardness of habitual diet, the results of the present study should be interpreted with great caution. Nevertheless, our findings should provide valuable insights into this poorly explored research issue.

Furthermore, misreporting of food intake, particularly by overweight persons, is a serious problem in self-reported dietary assessment methods (24). Consistent misreporting across all types of foods likely has little influence on energy-adjusted dietary hardness values (25), but studies indicate that overweight persons may selectively underreport their intakes of fatty or sugary foods (26, 27), which could cause dietary hardness estimations to be higher than actual values. In the present study, the potential shared error created by underreporting of dietary measures by subjects with a high BMI (and WC) would likely have weakened the associations of dietary hardness with measures of obesity and could possibly have led to a null finding; this possibility may at least partly explain the lack of association with BMI. Nonetheless, we did find a significant negative association with WC.

Finally, although we attempted to adjust for a wide range of potential confounding variables, we could not rule out residual confounding. Physical activity in particular was assessed relatively roughly from only 5 different activities, a number that may not have been sufficient. In addition, whereas dental status has an influence on food and nutrient intakes and on obesity (28–30), particularly in older persons, we unfortunately had no information on the subjects' dental status, which could confound the present results for young women. Although impaired dental status may be less pervasive in young than in elderly populations, and although the percentage of subjects in a similar population (3828 Japanese female dietetics students aged 18–20 y) who had been diagnosed by a dentist as having a dental disease was relatively small (8%) (S Sasaki, unpublished observations, 2007), further research on dietary hardness and health should take the subjects' dental status into account.

In conclusion, the results of the present study showed that, whereas there was no association between dietary hardness and BMI, dietary hardness was a significant independent determinant of WC in a group of free-living young Japanese women. Because these observations are generally consistent with the results of several animal studies (6–9), the present findings could make innovative contributions to the literature and raise issues for future studies on diet and obesity. However, because this is a preliminary study with a novel, as yet unestablished method of assessing dietary hardness, the results should be interpreted with great caution; nevertheless, applications of the method of assessing dietary hardness to other similar datasets would be of some interest. To better understand the influence of dietary hardness on obesity, further observational and intervention studies are clearly needed. To conduct such investigations, it is urgent to develop a database of values for a variable indicating hardness (eg, masticatory muscle activity) of various food items.

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The author's responsibilities were as follows—KMurakami: contributed to the concept and design of the study, the study protocol, data management, and coordinated the field work, calculated the dietary hardness, analyzed and interpreted the data, and wrote the manuscript; SS: the concept and design of the study, the study protocol, and data management, and contributed to the writing and editing of the manuscript; YT: the writing and editing of the manuscript; KU: the concept and design of the study, the study protocol, and data collection; MY, HH, TG, JO, KB, KO, TK, KMuramatsu, and MF: data collection. All authors contributed to the preparation of the manuscript and approved the final version submitted for publication. None of the authors had any personal or financial conflict of interest.

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Nutrient and food intake in relation to serum leptin concentration among young Japanese women

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Abstract

Objective: Little is known about the relation of modifiable dietary factors to circulating leptin concentrations, particularly in young adults and non-Western populations. We examined cross-sectional associations between nutrient and food intake and serum leptin concentration in young Japanese women.

Methods: Subjects were 424 female Japanese dietetic students 18–22 y of age. Intake of macronutrients (protein, total fat; saturated, monounsaturated, and polyunsaturated fatty acids; and carbohydrate), dietary fiber, and 12 food groups was assessed with a validated, self-administered, comprehensive, diet history questionnaire. Fasting blood samples were collected, and serum leptin concentrations were measured by radioimmunoassay.

Results: For nutrients, only dietary fiber was a significant determinant of serum leptin concentration. Increasing dietary fiber intake was associated with lower serum leptin concentration independent of potential confounding factors, including body mass index (mean serum leptin concentrations in the lowest and highest quintiles of dietary fiber intake were 8.6 and 7.5 ng/mL, respectively; P for trend = 0.026). Vegetables and pulses were the only foods significantly associated with serum leptin concentration, with higher intakes independently associated with lower concentrations (mean serum leptin concentrations in the lowest and highest quintiles of intake were 8.1 and 7.0 ng/mL, P for trend = 0.007, for vegetables and 8.8 and 7.6 ng/mL, P for trend = 0.019, for pulses, respectively).

Conclusion: Intake of dietary fiber, vegetables, and pulses showed an independent inverse association with serum leptin concentration in a group of young Japanese women.

Keywords: Dietary fiber; Vegetables; Pulses; Leptin; Japanese women; Epidemiology.

Introduction

Circulating leptin concentrations are highly positively correlated with body mass index (BMI) [1]. Despite this strong association, levels show large individual variation for a given level of adiposity [1], indicating the likely affect of variables other than adipose mass, such as genetic and environmental factors. Given the potential for positive associations of leptin concentration with subsequent weight gain [2] and the development of cardiovascular disease [3,4], the identification of modifiable lifestyle factors asso-

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ciated with leptin levels, e.g., dietary habits, is vitally important from a prevention perspective [5].

Relatively little is known about the effect of specific dietary factors on circulating serum levels [6]. Although several Western studies have failed to find significant associations between energy-providing nutrient intake and circulating leptin concentrations [7,8], total fat and polyunsaturated fatty acid intakes were significantly positively associated with plasma leptin level in middle-aged American men [9]. At the food level, a favorable effect of whole grains [10], vegetables [11], and fish [12] has been suggested in Western studies. However, evidence from people in non-Western countries and young adult populations is limited [7–12]. We conducted a cross-sectional study of associations between nutrient and food intake and serum leptin concentration in a group of young Japanese women.

Materials and methods

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. 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 general purpose and procedure of the survey. A total of 474 women took part. 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 years old. For the present analysis, we selected 424 women who met the following five inclusion criteria: age 18–22 y (n = 467), not currently receiving dietary counseling from a doctor or a dietitian (n = 468), having a reported energy intake within 1000–3500 kcal/d (n = 467), able to provide a fasting blood sample (n = 465), and having measured serum leptin concentrations (n = 452).

Dietary habits during the preceding month were assessed using a previously validated, self-administered, comprehensive, diet history questionnaire (DHQ) [13–15]. Responses to the DHQ and those to an accompanying lifestyle questionnaire were checked at least twice for completeness. When necessary, forms were reviewed with the subject 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 [16]. Pearson's correlation coefficients between the DHQ and 3-d estimated dietary records were 0.48 for protein, 0.55 for total fat, 0.75 for saturated fatty acid, 0.50 for monounsaturated fatty acid, 0.37 for polyunsaturated fatty acid, and 0.48 for carbohydrate in 47 women [13]. In addition, Pearson's correlation coefficients between the DHQ and 16-d semiweighed dietary records were 0.48 for protein, 0.60 for total fat, 0.71 for saturated fatty acid, 0.55 for monounsaturated fatty acid, 0.34 for polyunsaturated fatty acid, 0.64 for carbohydrate, and 0.70 for dietary fiber in 92 women, and the mean value of Spearman's correlation coefficients of food groups was 0.44, with a range of 0.13 to 0.77 (unpublished observations, S. Sasaki, 2006).

About 1–3 d after completion of the questionnaires, blood was sampled after an overnight fast in evacuated tubes containing no additives, allowed to clot, and centrifuged at 3000g for 10 min at room temperature to separate serum. According to the survey protocol, blood samples were transported at −20°C by car or airplane to ensure delivery to a laboratory in Tokyo (SRL Inc., Tokyo, Japan) within 2 d of collection to avoid significant degradation. Serum leptin concentrations were measured at SRL by radioimmunoassay. In-house quality-control procedures were fulfilled at SRL. The within- and between-assay coefficients of variation were 3.5% and 4.2%, respectively.

Body height was measured to the nearest 0.1 cm with the subject standing without shoes. Body weight in light indoor clothing was measured to the nearest 0.1 kg. BMI 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 (north: Kanto and Tohoku, central: Tokai and Hokuriku, or south: Kyushu and Chugoku) and into three categories according to population size (city with population ≥1 million, city with population <1 million, or town and village). Current smoking status (yes or no) was self-reported in the lifestyle questionnaire. Rate of eating (slow, medium, or fast) was self-reported as part of the DHQ. Alcohol drinking was assessed using the DHQ and grouped into three categories (non-drinker, >0% to <1% energy, or ≥1% energy). Physical activity was computed as average metabolic equivalent-hours [17] 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 reported in the lifestyle questionnaire.

Associations with serum leptin concentration were examined for the intake of selected nutrients including protein, total fat, saturated, monounsaturated, and polyunsaturated fatty acids, carbohydrate, and dietary fiber and 12 food groups (cereals, potatoes, confectioneries, fats and oils, fruits, vegetables including mushrooms and seaweeds, pulses including nuts, meats, eggs, fish and shellfish, dairy products, and beverages). We used energy-adjusted values of dietary intake, i.e., percentage of energy from protein, fat including fatty acids, and carbohydrate and amounts (grams) per 1000 kcal of energy for dietary fiber and foods. For analyses, subjects were categorized into quintiles according to dietary intake. Mean values (95% confidence intervals) for serum leptin concentration were calculated after multivariate adjustment for potential confounding fac-

tors, including residential block, size of residential area, current smoking, rate of eating, alcohol drinking, physical activity (total metabolic equivalent-hours/day, continuous), and energy intake (kilocalories/day, continuous; model 1). We also included BMI (kilograms per square meter, continuous) as a confounding factor (model 2). For nutrients, we further adjusted for intake (continuous) of other nutrients (total fat as percentage of energy and dietary fiber as grams per 1000 kcal for protein; protein as percentage of energy and dietary fiber for total fat, saturated, monounsaturated, polyunsaturated fatty acids, and carbohydrate; and protein and total fat for dietary fiber; model 3). For fatty acids, we further adjusted for other fatty acids (model 4). Because the distribution of serum leptin concentrations was highly skewed, the analyses were done using natural log-transformed values. We tested for linear trends with increasing levels of dietary intake by assigning each participant the median value for the category and modeling this value as a continuous variable. All statistical analyses were performed using SAS 8.2 (SAS Institute, Cary, NC, USA). All reported P values are two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Subject characteristics are listed in Table 1. Mean serum leptin concentration was 7.7 ng/mL. Mean intakes of protein, fat, and carbohydrate were 13.9%, 29.6%, and 55.1% energy, respectively, whereas mean intakes of dietary fiber, vegetables, and pulses were 7.1, 129.4, and 25.3 g/1000 kcal, respectively. Subject characteristics according to quintile of serum leptin concentration are also presented in Table 1. Women in the higher quintiles of serum leptin weighed more, consumed less alcohol, and had lower intake of protein, dietary fiber, vegetables, and pulses.

Independent associations between nutrient intake and serum leptin concentration are presented in Table 2. Intakes of protein and polyunsaturated fatty acids were significantly inversely associated with serum leptin concentration, independent of not only potential confounding factors (model 1: P for trend = 0.004 and 0.008, respectively) but also of BMI (model 2: P for trend = 0.007 and 0.035, respectively). This association disappeared after further adjustment for intake of other nutrients (model 3: P for trend = 0.21 and 0.23, respectively; model 4 for polyunsaturated fatty acids: P for trend = 0.86). Although total fat intake was significantly inversely associated with serum leptin concentration, independent of potential confounding factors (model 1: P for trend = 0.033), this was not independent of BMI (model 2: P for trend = 0.23) or other nutrients (model 3: P for trend = 0.31). Intakes of saturated and monounsaturated fatty acids and carbohydrate were not significantly associated with serum leptin concentration regardless of adjustment for other factors. Conversely, dietary fiber intake was a significant determinant of serum leptin concentration, independent of not only potential confounding factors (model 1: P for trend = 0.0003) including BMI (model 2: P for trend = 0.003) but also of other nutrient intakes (model 3: P for trend = 0.026). Higher dietary fiber intake was independently associated with lower serum leptin concentration (mean difference in serum leptin concentration between the lowest and highest quintiles of dietary fiber intake = −1.1 ng/mL, model 3).

Table 3 presents independent associations between food intake and serum leptin concentration. Cereals were significantly positively associated with serum leptin concentration, independent of potential confounding factors (model 1: P for trend = 0.013), but this was not independent of BMI (model 2: P for trend = 0.22). Vegetables and pulses were significantly associated with serum leptin concentration independently of not only potential confounding factors (model 1: P for trend = 0.001 and 0.027, respectively) but also of BMI (model 2: P for trend = 0.007 and 0.019, respectively). Increasing intake of these foods was independently associated with a lower serum leptin concentration (mean difference in serum leptin concentration between the lowest and highest quintiles of intake = −1.1 ng/mL for vegetables and −1.2 ng/mL for pulses, model 2). However, after further adjustment for intake of dietary fiber, a single nutrient independently associated with serum leptin concentration in the present study, the significant associations of intake of vegetables and pulses with serum leptin concentration disappeared (P for trend = 0.50 and 0.25, respectively). Intakes of other foods were not significantly associated with serum leptin concentration regardless of adjustment for other factors.

Discussion

In this study of young Japanese women, we found that higher intakes of dietary fiber, vegetables, and pulses were associated with lower serum leptin concentrations, independently of potential confounding factors including BMI. Given that our subjects were selected female dietetic students rather than a random sample of Japanese women, these results might not be extrapolated to the general Japanese population. However, the biological relation between diet and leptin levels in this population is likely similar to that among women in general. In addition, because the study population consisted of generally healthy participants, the clinical relevance of our findings remains to be elucidated. Nevertheless, our results should provide valuable insight from a prevention perspective. The relatively healthy dietary habits and the narrower range of leptin levels in this population of young healthy and lean women would mean that greater differences might be seen in other populations.

On average, serum leptin concentration in this study of 18- to 22-y-old Japanese women with a mean BMI of 21.4 kg/m^2 (arithmetic mean = 8.8 ng/mL) was relatively com-

Table 1
Subject characteristics according to quintile of serum leptin concentration*

Variable	All (n = 424)	Quintile of serum leptin concentration					$P^{\dagger}$
		1 (n = 89)	2 (n = 80)	3 (n = 86)	4 (n = 83)	5 (n = 86)	
Serum leptin concentration (ng/mL)	7.7 (7.3–8.1)	4.2	5.9	7.8	10.4	14.5	
Age (y)	19.5 ± 1.0	19.9 ± 1.2	19.5 ± 0.8	19.3 ± 0.9	19.3 ± 1.0	19.4 ± 0.9	0.002
Body height (cm)	157.9 ± 5.6	158.4 ± 5.3	157.8 ± 5.1	156.7 ± 6.0	158.7 ± 6.0	157.8 ± 5.3	0.91
Body weight (kg)	53.3 ± 8.1	48.1 ± 4.4	50.2 ± 4.8	51.8 ± 5.4	55.2 ± 5.1	61.4 ± 10.9	<0.0001
Body mass index (kg/m^2)	21.4 ± 2.9	19.2 ± 1.6	20.1 ± 1.6	21.1 ± 1.8	21.9 ± 1.7	24.6 ± 3.8	<0.0001
Residential block							0.038
North (Kanto and Tohoku)	263 (62)	47 (53)	43 (54)	61 (71)	57 (69)	55 (64)	
Central (Tokai and Hokuriku)	68 (16)	17 (19)	17 (21)	10 (12)	11 (13)	13 (15)	
South (Kyushu and Chugoku)	93 (22)	25 (28)	20 (25)	15 (17)	15 (18)	18 (21)	
Size of residential area							0.23
City with population ≥1 million	75 (18)	15 (17)	15 (19)	21 (24)	12 (15)	12 (14)	
City with population <1 million	314 (74)	66 (74)	61 (76)	60 (70)	64 (77)	63 (73)	
Town and village	35 (8)	8 (9)	4 (5)	5 (6)	7 (8)	11 (13)	
Current smoking							0.55
No	412 (97)	86 (97)	76 (95)	86 (100)	80 (96)	84 (98)	
Yes	12 (3)	3 (3)	4 (5)	0 (0)	3 (4)	2 (2)	
Rate of eating							0.013
Slow	134 (32)	32 (36)	28 (35)	29 (34)	19 (23)	26 (30)	
Medium	134 (32)	30 (34)	26 (33)	29 (34)	33 (40)	16 (19)	
Fast	156 (37)	27 (30)	26 (33)	28 (33)	31 (37)	44 (51)	
Alcohol drinking							0.033
Non-drinker	253 (60)	47 (53)	39 (49)	58 (67)	53 (64)	56 (65)	
>0% to <1% energy	103 (24)	25 (28)	22 (28)	22 (26)	17 (20)	17 (20)	
≥1% energy	68 (16)	17 (19)	19 (24)	6 (7)	13 (16)	13 (15)	
Physical activity (total metabolic equivalents-h/d)	34.1 ± 3.5	34.1 ± 3.3	34.0 ± 3.0	34.5 ± 4.8	34.5 ± 3.9	33.4 ± 2.1	0.27
Energy intake (kcal/d)	1764 ± 408	1715 ± 417	1831 ± 420	1775 ± 373	1723 ± 397	1782 ± 429	0.83
Nutrient intake							
Protein (% energy)	13.9 ± 1.9	14.3 ± 2.0	13.8 ± 1.9	14.1 ± 1.8	13.8 ± 1.7	13.4 ± 1.8	0.003
Total fat (% energy)	29.6 ± 5.0	30.0 ± 5.0	30.1 ± 5.1	29.7 ± 4.2	28.6 ± 4.9	29.4 ± 5.5	0.18
Saturated fatty acids (% energy)	8.6 ± 2.0	8.6 ± 1.8	8.9 ± 2.2	8.6 ± 1.8	8.4 ± 2.1	8.5 ± 2.1	0.29
Monounsaturated fatty acids (% energy)	10.2 ± 2.1	10.4 ± 2.1	10.4 ± 2.0	10.2 ± 1.9	9.9 ± 2.0	10.1 ± 2.4	0.13
Polyunsaturated fatty acids (% energy)	6.5 ± 1.3	6.6 ± 1.2	6.5 ± 1.2	6.5 ± 1.3	6.4 ± 1.4	6.3 ± 1.6	0.071
Carbohydrate (% energy)	55.1 ± 5.7	54.3 ± 5.7	54.6 ± 5.8	55.0 ± 4.9	55.8 ± 5.7	55.5 ± 6.4	0.089
Dietary fiber (g/1000 kcal)	7.1 ± 2.1	7.6 ± 2.5	7.3 ± 2.2	7.1 ± 1.8	7.0 ± 2.1	6.6 ± 1.8	0.0009
Food intake (g/1000 kcal)							
Cereals	215.7 ± 55.5	206.9 ± 52.3	210.5 ± 51.5	214.1 ± 49.9	229.1 ± 61.7	218.4 ± 59.8	0.052
Potatoes	18.2 ± 12.9	19.7 ± 12.2	16.4 ± 11.9	19.1 ± 13.6	19.0 ± 12.8	16.6 ± 13.6	0.32
Confectioneries	50.4 ± 21.2	50.2 ± 20.9	50.0 ± 20.3	49.4 ± 21.8	45.1 ± 16.7	57.0 ± 24.2	0.079
Fats and oils	11.8 ± 5.0	12.3 ± 5.2	11.8 ± 4.4	11.9 ± 4.8	11.5 ± 5.0	11.4 ± 5.3	0.22
Fruits	35.7 ± 33.9	36.9 ± 33.1	35.3 ± 30.4	38.4 ± 38.1	32.3 ± 30.7	35.2 ± 36.6	0.61
Vegetables[‡]	121.8 ± 72.4	138.7 ± 87.1	126.5 ± 80.3	121.7 ± 58.8	119.8 ± 59.7	101.8 ± 68.1	0.0008
Pulses[§]	25.3 ± 16.4	26.7 ± 17.9	26.1 ± 16.7	25.9 ± 14.4	26.5 ± 16.7	21.5 ± 15.6	0.040
Meats	33.2 ± 16.2	35.6 ± 17.9	34.2 ± 18.7	31.1 ± 13.6	33.8 ± 13.3	31.5 ± 16.9	0.15
Eggs	20.9 ± 12.8	21.9 ± 12.3	18.3 ± 10.7	24.5 ± 14.5	18.7 ± 11.2	20.9 ± 14.1	0.69
Fish and shellfish	29.7 ± 15.5	32.6 ± 17.2	29.4 ± 15.4	29.5 ± 16.6	29.6 ± 13.6	27.4 ± 14.3	0.052
Dairy products	89.2 ± 75.8	84.5 ± 62.4	86.0 ± 70.7	99.8 ± 74.1	99.7 ± 94.0	76.4 ± 73.9	0.58
Beverages	411.8 ± 254.8	448.4 ± 272.1	409.4 ± 230.0	392.1 ± 253.8	410.8 ± 253.6	397.0 ± 262.4	0.29

* Values are means ± standard deviations for continuous variables and number of subjects (%) for categorical variables except for serum leptin concentration (geometric mean [95% confidence interval] for total sample and median for each quintile).

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

‡ Including mushrooms and sea vegetables.

§ Including nuts.

Table 2
Serum leptin concentration (mg/ml) according to quintile of nutrient intake (n = 424)*

Variable	Quintile of nutrient intake					P for trend†
	1 (n = 84)	2 (n = 85)	3 (n = 85)	4 (n = 85)	5 (n = 85)	
Protein (% energy)	11.7	12.8	13.8	14.6	16.3	
Model 1‡	8.8 (7.8–9.8)	8.1 (7.2–9.1)	7.4 (6.6–8.3)	7.2 (6.4–8.1)	7.1 (6.3–7.9)	0.004
Model 2§	8.5 (7.8–9.3)	7.8 (7.1–8.5)	7.5 (6.9–8.1)	7.6 (7.0–8.3)	7.1 (6.5–7.8)	0.007
Model 3‖	8.2 (7.5–9.0)	7.6 (7.0–8.3)	7.4 (6.8–8.1)	7.7 (7.1–8.4)	7.4 (6.8–8.1)	0.21
Total fat (% energy)	23.5	27.1	29.6	31.9	36.0	
Model 1‡	8.6 (7.6–9.7)	7.5 (6.7–8.5)	7.6 (6.7–8.5)	7.9 (7.0–8.9)	6.9 (6.1–7.8)	0.033
Model 2§	8.0 (7.3–8.7)	7.6 (7.0–8.3)	7.7 (7.1–8.4)	7.9 (7.3–8.6)	7.2 (6.6–7.9)	0.23
Model 3¶	7.9 (7.2–8.6)	7.6 (7.0–8.3)	7.8 (7.2–8.5)	7.9 (7.2–8.6)	7.2 (6.6–7.9)	0.31
Saturated fatty acids (% energy)	6.3	7.5	8.3	9.4	11.4	
Model 1‡	8.5 (7.5–9.5)	7.4 (6.6–8.4)	7.4 (6.6–8.3)	7.7 (6.8–8.6)	7.5 (6.6–8.4)	0.29
Model 2§	8.1 (7.4–8.8)	7.3 (6.7–7.9)	7.6 (7.0–8.3)	8.0 (7.4–8.7)	7.4 (6.8–8.1)	0.55
Model 3¶	8.1 (7.4–8.8)	7.4 (6.8–8.0)	7.7 (7.0–8.3)	8.0 (7.4–8.7)	7.3 (6.7–8.0)	0.37
Model 4**	7.8 (7.0–8.7)	7.3 (6.6–7.9)	7.6 (7.0–8.3)	8.1 (7.5–8.9)	7.6 (6.8–8.5)	0.88
Monounsaturated fatty acids (% energy)	7.6	9.0	10.1	11.2	13.0	
Model 1‡	8.4 (7.5–9.4)	7.7 (6.9–8.7)	7.8 (6.9–8.7)	7.3 (6.5–8.2)	7.3 (6.5–8.2)	0.08
Model 2§	8.1 (7.4–8.8)	7.7 (7.1–8.4)	7.6 (7.0–8.3)	7.5 (6.9–8.1)	7.5 (6.9–8.2)	0.26
Model 3¶	8.0 (7.3–8.8)	7.7 (7.1–8.4)	7.7 (7.1–8.4)	7.5 (6.9–8.1)	7.5 (6.9–8.2)	0.26
Model 4††	7.7 (6.7–8.8)	7.6 (6.9–8.3)	7.7 (7.1–8.4)	7.6 (6.9–8.3)	7.9 (6.8–9.0)	0.85
Polyunsaturated fatty acids (% energy)	4.9	5.7	6.4	7.1	8.1	
Model 1‡	9.0 (8.0–10.1)	7.9 (7.1–8.9)	7.4 (6.6–8.3)	6.7 (6.0–7.5)	7.5 (6.7–8.4)	0.01
Model 2§	8.5 (7.8–9.2)	7.8 (7.1–8.5)	7.5 (6.9–8.2)	7.2 (6.6–7.8)	7.5 (6.9–8.2)	0.035
Model 3¶	8.3 (7.5–9.0)	7.7 (7.1–8.4)	7.5 (6.9–8.2)	7.2 (6.6–7.9)	7.7 (7.1–8.4)	0.23
Model 4‡‡	8.0 (7.0–9.2)	7.6 (6.9–8.4)	7.5 (6.9–8.2)	7.3 (6.7–8.0)	8.0 (6.9–9.1)	0.86
Carbohydrate (% energy)	47.8	52.4	55.0	57.8	62.4	
Model 1‡	7.5 (6.7–8.5)	7.5 (6.6–8.4)	7.2 (6.4–8.0)	7.8 (6.9–8.7)	8.5 (7.6–9.6)	0.14
Model 2§	7.5 (6.9–8.2)	7.6 (7.0–8.3)	7.6 (6.9–8.2)	7.7 (7.1–8.4)	7.9 (7.3–8.7)	0.38
Model 3¶	7.7 (6.9–8.4)	7.7 (7.0–8.4)	7.6 (7.0–8.3)	7.7 (7.1–8.4)	7.8 (7.0–8.6)	0.84
Dietary fiber (g/1000 kcal)	5.0	6.0	6.8	7.8	9.7	
Model 1‡	9.1 (8.1–10.2)	8.4 (7.5–9.4)	7.1 (6.4–8.0)	6.9 (6.2–7.8)	7.0 (6.3–7.9)	0.0003
Model 2§	8.7 (8.0–9.4)	8.0 (7.3–8.7)	7.5 (6.9–8.2)	7.0 (6.4–7.6)	7.4 (6.8–8.0)	0.003
Model 3§§	8.6 (7.9–9.4)	7.9 (7.3–8.6)	7.5 (6.9–8.1)	7.0 (6.5–7.7)	7.5 (6.8–8.2)	0.026

* Values are medians for nutrient intake and geometric means (95% confidence intervals) for serum leptin concentration.

† Tests for linear trend used the median value in each quintile as a continuous variable in linear regression.

‡ Adjusted for residential block (north: Kanto and Tohoku, central: Tokai and Hokuriku, and 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), rate of eating (slow, medium, or fast), alcohol drinking (non-drinker, >0% to <1% energy, or ≥1% energy), physical activity (total metabolic equivalents-hours/day, continuous), and energy intake (kcal/d, continuous).

§ Adjusted for variables used in model 1 and body mass index (kg/m^2, continuous).

‖ Adjusted for variables used in model 2 and intakes (continuous) of total fat (% energy) and dietary fiber (g/1000 kcal).

¶ Adjusted for variables used in model 2 and intakes (continuous) of protein (% energy) and dietary fiber (g/1000 kcal).

** Adjusted for variables used in model 3 and intakes (continuous) of monounsaturated fatty acids (% energy) and polyunsaturated fatty acids (% energy).

†† Adjusted for variables used in model 3 and intakes (continuous) of saturated fatty acids (% energy) and polyunsaturated fatty acids (% energy).

‡‡ Adjusted for variables used in model 3 and intakes (continuous) of saturated fatty acids (% energy) and monounsaturated fatty acids (% energy).

§§ Adjusted for variables used in model 2 and intakes (continuous) of protein (% energy) and total fat (% energy).

parable to those in a limited number of studies of lean young women. Arithmetic mean values of circulating leptin level were 8.2 ng/mL in 63 Canadian women aged 18–35 y (mean BMI = 21.9 kg/m^2) [18], 9.1 ng/mL in 18 American women aged 20–31 y (mean BMI = 21.9 kg/m^2) [7], and 9.9 ng/mL in 61 Greek women aged 14–26 y (mean BMI = 21.2 kg/m^2) [8]. Our mean estimate of dietary fiber intake was 12.6 g/d, which was comparable to that in a representative sample of Japanese women aged 18–29 y (12.0 g/d) [19].

Intakes of protein, fat (including fatty acids), and carbohydrate were not independently associated with serum leptin concentration in the present study. Although protein and polyunsaturated fatty acid intake was inversely associated with serum leptin concentration independent of potential confounding factors including BMI, this association was not independent of other nutrient intake. This may be due to relatively strong positive correlations of intakes of protein and polyunsaturated fatty acids with dietary fiber intake, a single nutrient independently associated with serum leptin concentration in the present study (Pearson's correlation coefficient, r = 0.42 and 0.20, respectively) compared with those of other nutrients (r = −0.15 to −0.01). No association between macronutrient intake and circulating leptin

Table 3
Serum leptin concentration (mg/ml) according to quintile of food intake (n = 424)*

Variable	Quintile of nutrient intake					P for trend†
	1 (n = 84)	2 (n = 85)	3 (n = 85)	4 (n = 85)	5 (n = 85)	
Cereals (g/1000 kcal)	144.8	187.4	214.2	240.8	285.3	
Model 1‡	7.3 (6.5–8.2)	7.1 (6.3–8.0)	7.4 (6.6–8.3)	7.7 (6.8–8.6)	9.0 (8.0–10.2)	0.013
Model 2‡§	7.8 (7.1–8.5)	7.3 (6.7–8.0)	7.1 (6.6–7.8)	7.9 (7.3–8.6)	8.3 (7.6–9.0)	0.22
Potatoes (g/1000 kcal)	6.2	10.8	15.1	20.9	33.7	
Model 1‡	8 (7.1–8.9)	7.8 (6.9–8.7)	8.5 (7.6–9.6)	6.9 (6.2–7.8)	7.3 (6.5–8.1)	0.12
Model 2‡§	8.0 (7.3–8.7)	7.6 (7.0–8.3)	7.8 (7.1–8.5)	7.3 (6.7–7.9)	7.8 (7.2–8.5)	0.70
Confectioneries (g/1000 kcal)	26.9	39.1	46.8	57.0	77.8	
Model 1‡	7.3 (6.5–8.2)	7.6 (6.8–8.5)	8.3 (7.5–9.3)	6.8 (6.1–7.6)	8.4 (7.5–9.4)	0.25
Model 2‡§	7.6 (7.0–8.3)	7.2 (6.6–7.8)	8.0 (7.4–8.7)	7.3 (6.7–7.9)	8.3 (7.6–9.0)	0.15
Fats and oils (g/1000 kcal)	6.0	8.8	11.1	14.1	17.8	
Model 1‡	7.9 (7.1–8.9)	8.2 (7.3–9.1)	7.9 (7.1–8.9)	7.2 (6.4–8.0)	7.2 (6.4–8.1)	0.07
Model 2‡§	7.9 (7.3–8.6)	7.9 (7.3–8.6)	7.7 (7.1–8.4)	7.5 (6.9–8.2)	7.3 (6.7–8.0)	0.14
Fruits (g/1000 kcal)	7.8	16.0	25.1	40.6	75.2	
Model 1‡	8.7 (7.8–9.8)	7.4 (6.6–8.3)	8.1 (7.2–9.1)	6.8 (6.1–7.7)	7.4 (6.6–8.3)	0.09
Model 2‡§	8.5 (7.8–9.3)	7.4 (6.8–8.1)	7.9 (7.3–8.6)	7.0 (6.5–7.6)	7.6 (7.0–8.3)	0.13
Vegetables (g/1000 kcal)‖	47.5	79.3	105.6	139.0	211.0	
Model 1‡	8.5 (7.6–9.5)	8.5 (7.6–9.5)	7.3 (6.6–8.2)	7.6 (6.8–8.5)	6.6 (5.9–7.4)	0.001
Model 2‡§	8.1 (7.5–8.9)	8.1 (7.5–8.8)	7.6 (6.9–8.2)	7.7 (7.1–8.4)	7.0 (6.4–7.6)	0.007
Pulses (g/1000 kcal)¶	8.5	15.2	21.2	30.5	46.9	
Model 1‡	9.0 (8.0–10.0)	8.1 (7.3–9.1)	6.9 (6.2–7.7)	7.2 (6.4–8.0)	7.4 (6.6–8.3)	0.027
Model 2‡§	8.8 (8.1–9.6)	7.9 (7.3–8.6)	7.1 (6.6–7.8)	7.1 (6.5–7.7)	7.6 (7.0–8.2)	0.019
Meats (g/1000 kcal)	15.8	23.7	30.0	39.9	52.4	
Model 1‡	8.1 (7.2–9.0)	7.4 (6.7–8.3)	7.8 (7.0–8.7)	7.9 (7.1–8.9)	7.2 (6.4–8.0)	0.32
Model 2‡§	8.1 (7.4–8.8)	7.4 (6.8–8.0)	7.6 (7.0–8.3)	7.8 (7.2–8.5)	7.5 (6.9–8.2)	0.53
Eggs (g/1000 kcal)	4.7	13.1	21.3	27.5	35.5	
Model 1‡	8.4 (7.5–9.4)	7.5 (6.7–8.4)	7.4 (6.6–8.3)	7.4 (6.6–8.3)	7.8 (7.0–8.7)	0.33
Model 2‡§	8.2 (7.5–8.9)	7.2 (6.6–7.8)	7.5 (6.9–8.2)	7.6 (7.0–8.3)	7.9 (7.2–8.6)	0.83
Fish and shellfish (g/1000 kcal)	12.3	21.0	27.2	35.2	50.6	
Model 1‡	8.3 (7.4–9.3)	7.5 (6.7–8.4)	7.8 (7.0–8.8)	7.6 (6.8–8.5)	7.2 (6.4–8.1)	0.14
Model 2‡§	8.1 (7.4–8.8)	7.7 (7.1–8.4)	7.8 (7.2–8.5)	7.5 (6.9–8.2)	7.3 (6.7–7.9)	0.08
Dairy products (g/1000 kcal)	13.9	37.7	69.1	110.0	210.8	
Model 1‡	8.2 (7.3–9.2)	8.0 (7.1–8.9)	7.3 (6.5–8.2)	7.3 (6.5–8.2)	7.7 (6.8–8.6)	0.46
Model 2‡§	8.5 (7.8–9.2)	7.7 (7.1–8.4)	7.1 (6.6–7.7)	7.3 (6.7–7.9)	7.9 (7.2–8.6)	0.52
Beverages (g/1000 kcal)	119.8	267.4	355.7	505.8	767.8	
Model 1‡	8.2 (7.4–9.2)	7.1 (6.3–8.0)	8.1 (7.3–9.1)	7.4 (6.6–8.3)	7.6 (6.8–8.5)	0.46
Model 2‡§	8.2 (7.6–9.0)	7.5 (6.9–8.1)	7.9 (7.2–8.5)	7.3 (6.7–7.9)	7.6 (7.0–8.3)	0.22

* Values are medians for food intake and geometric means (95% confidence intervals) for serum leptin concentration.

† Tests for linear trend used the median value in each quintile as a continuous variable in linear regression.

‡ Adjusted for residential block (north: Kanto and Tohoku, central: Tokai and Hokuriku, and 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), rate of eating (slow, medium, or fast), alcohol drinking (non-drinker, >0% to <1% energy, or ≥1% energy), physical activity (total metabolic equivalents-hours/day, continuous), and energy intake (kcal/d, continuous).

§ Further adjusted for body mass index (kg/m^2, continuous).

‖ Including mushrooms and sea vegetables.

¶ Including nuts.

level was obtained in 32 American men and women aged 20–31 y [7] and 114 Greek men and women aged 14–26 y [8]. In contrast, although macronutrient composition was not independently associated with plasma leptin concentrations in 268 American men aged 47–83 y, the percentage of energy from total fat and monounsaturated fat showed an independent positive association in a subanalysis of 121 men with a BMI <25 kg/m^2 [9]. These discrepancies might be explained at least in part by the different populations investigated, different dietary assessment methods used, differences in the number and type of variables used as confounding factors, whether or not other nutrients in multivariate analysis were taken into account. Further studies are needed in this poorly investigated field.

Dietary fiber intake showed an independent inverse association with serum leptin concentration. At the food level, an independent negative association was seen between the intake of vegetables and pulses and serum leptin concentration. The finding on vegetables and pulses is very consistent with the finding on dietary fiber, because vegetables and pulses were the top and second contributors to dietary fiber in the present study (39.1% and 11.4%), respectively, and the significant associations of vegetables and pulses with serum leptin disappeared after further adjustment for dietary

fiber. To our knowledge, no previous study has investigated an association between circulating leptin level and dietary fiber and its main sources such as vegetables and pulses. However, a study in 938 middle-aged American men and women found that the intake of whole grains, which are high in dietary fiber, showed an independent inverse association with plasma leptin levels [10]. We were unable to investigate this association because only 7% of our subjects reported the consumption of whole grains. In addition, a Polish study found that vegetarian prepubertal children (n = 22) had a significantly higher dietary fiber intake and significantly lower serum leptin concentrations than did control omnivores (n = 13) [11]. These findings do not conflict with our results. An African population consuming fish as the main component of their diet (n = 279) had significantly lower plasma leptin levels than a population that consumed no fish at all but rather mainly maize and rice (n = 329) [12]. In the present study, fish and shellfish intake showed an inverse association with serum leptin concentration, although without statistical significance (P for trend = 0.08). Further research in a range of populations is required to elucidate the apparent influence of diet on circulating leptin levels.

It remains unknown whether dietary factors influence circulating leptin levels directly or indirectly. One possibility is that dietary factors (such as dietary fiber, vegetables, and pulses) are associated with a decrease in serum concentrations through decreased leptin production. Alternatively, they may increase leptin sensitivity, leading in turn to a subsequent decline in leptin production through unknown feedback mechanisms, a possibility suggested by interventional [20] and observational [9] studies of circulating leptin levels and physical activity.

Several limitations of our 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. Second, we used a self-administered semiquantitative dietary assessment questionnaire for dietary data collection [13–15]. Although the questionnaire had been previously validated, actual dietary habits were not observed, so the results should be interpreted cautiously. A population of young female dietetic students in particular would be expected to report the assumed "correct" and balanced dietary intake. However, mean reported intakes of energy, protein, total fat, and carbohydrate (1764 kcal/d, 61.4 g/d, 58.8 g/d, and 240.9 g/d, respectively) and mean percentages of energy from total fat and carbohydrate (29.6% and 55.1% energy, respectively) were relatively comparable to those in a representative sample of Japanese women aged 18–29 y (1701 kcal/d, 64.0 g/d, 56.0 g/d, 226.4 g/d, 29.1% energy, and 55.7% energy, respectively; not available for percentage of energy from protein) [19]. To minimize the influence of dietary under-reporting, an ongoing controversy in studies that collect dietary information using self-report instruments [21], we used energy-adjusted values of dietary intake. Third, single measurement of serum leptin concentration may represent short-term status only and introduce random errors. Nonetheless, this kind of error would tend to bias toward attenuating rather than enhancing the relation, and multiple or serial leptin measurements would have only increased the precision of the results. Fourth, although we attempted to adjust for a wide range of potential confounding variables, we could not rule out residual confounding. In particular, physical activity was assessed relatively roughly from only five activities, which might not have been sufficient. Furthermore, we could not adjust for body fat mass, which is strongly associated with circulating leptin levels [1], because of a lack of information on this variable in the present study, although we did adjust for BMI. Moreover, although leptin levels change during the menstrual cycle [22], we unfortunately did not assess menstrual cycle. Further research examining association of lifestyle factors with circulating leptin in reproductive-age women should take into account menstrual cycle.

Conclusion

Increasing intakes of dietary fiber, vegetables, and pulses were independently associated with lower serum leptin concentrations in young Japanese women. Because the cross-sectional nature of our study precludes causal inferences, any firm conclusions regarding the effect of diet on circulating leptin levels will require additional observational and experimental studies.

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ORIGINAL ARTICLE

Association between dietary fiber, water and magnesium intake and functional constipation among young Japanese women

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Objective: Most research on constipation has focused on dietary fiber intake. Here, we examined the intake of water and magnesium, nutrients possibly associated with constipation, as well as that of dietary fiber in relation to constipation.
Design: Cross-sectional study.
Subjects: A total of 3835 female Japanese dietetic students aged 18–20 years from 53 institutions in Japan.
Methods: Dietary intake was estimated with a validated, self-administered diet history questionnaire. Functional constipation was defined using the Rome I criteria.
Results: The prevalence of functional constipation was 26.2%. Neither dietary fiber intake (mean = 6.4 g/4186 kJ) nor intakes of total water and water from fluids were associated with constipation. Conversely, low intake of water from foods was associated with an increasing prevalence of constipation. In comparison with women in the first (lowest) quintile, the multivariate adjusted odds ratio (OR) (95% confidence interval (CI)) for women in the second, third, fourth, and fifth quintiles were 0.72 (0.57, 0.90), 0.78 (0.62, 0.98), 0.71 (0.56, 0.89), and 0.77 (0.61, 0.97), respectively (P for trend = 0.04). Additionally, low magnesium intake was associated with increasing prevalence of constipation. Compared with women in the first quintile, the multivariate adjusted OR (95% CI) for women in the second, third, fourth and fifth quintiles were 0.70 (0.56, 0.88), 0.75 (0.60, 0.95), 0.73 (0.58, 0.92) and 0.79 (0.63, 0.996), respectively (P for trend = 0.09).
Conclusions: Low intakes of water from foods and magnesium are independently associated with an increasing prevalence of functional constipation among a population whose dietary fiber intake is relatively low.
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Keywords: dietary fiber intake; water intake; magnesium intake; functional constipation; Japanese women; epidemiology

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Introduction

Constipation is a common health problem (Wong *et al.*, 1999; Pare *et al.*, 2001; Garrigues *et al.*, 2004; Higgins and Johanson, 2004), and diet is considered a major modifiable lifestyle factor associated with this condition (Locke *et al.*, 2000; Talley, 2004). The favorable effect of dietary fiber on constipation is widely accepted and several (Dukas *et al.*, 2003; Sanjoaquin *et al.*, 2004), although not all (Campbell *et al.*, 1993; Towers *et al.*, 1994; Murakami *et al.*, 2006), observational studies have indicated an inverse relation between dietary fiber intake and constipation. However, while most previous studies have defined constipation according to the infrequency of bowel movement (Campbell *et al.*, 1993; Towers *et al.*, 1994; Dukas *et al.*, 2003; Sanjoaquin

et al., 2004) or the subjective perception of patients (Murakami *et al.*, 2006), a consensus definition of constipation consists of straining, hard stools and incomplete evacuation in addition to infrequency (Rome criteria) (Whitehead *et al.*, 1991).

Other nutrients that might be associated with constipation include water and magnesium. Low intake of water can reduce the water content of stools and hence lead to constipation (Arnaud, 2003), although the potential benefit of an increase in intake is unknown (Klauser *et al.*, 1990; Anti *et al.*, 1998; Young *et al.*, 1998; Chung *et al.*, 1999). Magnesium might form sulfate or citrate salts that would promote fluid retention in the digestive tract and indirectly alter motility, and thereby act as a light laxative (Saez, 1991). To our knowledge, however, no observational studies have investigated the intake of water and magnesium in relation to constipation.

The aim of this cross-sectional study of young Japanese women was to examine associations between dietary fiber, water and magnesium intake, as assessed with a previously validated, self-administered diet history questionnaire (DHQ) (Sasaki *et al.*, 1998a, b, 2000), and functional constipation as defined according to the Rome criteria (Whitehead *et al.*, 1991).

Subjects and methods

Subjects and survey procedure

The study was based on a self-administered questionnaire survey of a wide range of dietary and non-dietary behaviors among dietetic students ($n=4679$) from 54 universities, colleges and technical schools in 33 of 47 prefectures in Japan. Staff at each institution distributed two questionnaires on dietary habits (DHQ) and other lifestyle items during the previous month to students during an orientation session or a first lecture designed for freshman students entering dietetic courses in April 2005; in most institutions, this was carried out within 2 weeks after the course began. Students filled out the questionnaires during the session, lecture, or at home and then submitted the completed forms to staff at each institution as soon as possible. A third questionnaire on lifestyle during the previous 6 years (i.e. junior high school and high school) was also distributed and answered in similar fashion; in most institutions, this was carried out within 4 weeks after the course began.

The staff at each institution checked the responses as soon as possible according to the survey protocol. When missing answers or logical errors were identified, the student was asked to complete the questionnaire again. The staff at each institution mailed the questionnaires to the survey center. Staff at the survey center checked the answers again and, when necessary, returned problematic questionnaires to staff at the respective institution, and the student was asked to complete the questionnaires again. All questionnaires were thus checked at least once by staff at the respective institution and by staff at the survey center. Most surveys were completed by May 2005. The protocol of the study was approved by the Ethics Committee of the National Institute of Health and Nutrition.

A total of 4286 students (4066 women and 220 men) answered all three questionnaires (response rate = 91.6%). For the purposes of the current analysis, we selected female subjects aged 18–20 years ($n=3967$). We then excluded from these 3967 women those who were in an institution where the survey had been conducted at the end of May ($n=97$), those with extremely low or high energy intake (<2093 or >16744 kJ/day) ($n=23$), and those with missing information on the variables studied ($n=24$). As some subjects were in more than one exclusion category, the final analysis sample comprised 3825 women. Further exclusion of subjects with intentional dietary change within the preceding year ($n=649$), those habitually using oral laxatives ($n=231$), or both did not materially alter the findings, and these were therefore included in the analyses.

Dietary intake

Dietary habits during the previous month were assessed using a previously validated, self-administered DHQ (Sasaki *et al.*, 1998a, b, 2000). This 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 semi-quantitative portion size of 121 selected food and non-alcoholic beverage items; dietary supplements; consumption frequency and semi-quantitative portion size of 19 staple foods (rice, bread and noodles) and miso soup (fermented soybean paste soup); and open-ended items for foods consumed regularly ($\geq$once/week) but not appearing in the DHQ. The food and beverage items and portion sizes in the DHQ were derived primarily from data in the National Nutrition Survey of Japan and several recipe books for Japanese dishes (Sasaki *et al.*, 1998a).

Estimates of dietary intake for 147 food and beverage items, energy, total, soluble and insoluble dietary fiber, total water, water from fluids, water from foods, and magnesium, were calculated using an *ad hoc* computer algorithm for the DHQ, which was based on the *Standard Tables of Food Composition in Japan* (Science and Technology Agency, 2000). Information on dietary supplements and data from the open-ended questionnaire items were not used in the calculation of dietary intake. Dietary fiber was determined by an enzymatic-gravimetric procedure (modified Prosky method) (Science and Technology Agency, 2000) from the intake of 86 fiber-containing foods in the DHQ. Total water was defined as the sum of water from all 147 food and beverage items. Water from fluids was defined as the sum of water from all beverages, milks, juices, and soups and water, whereas water from foods was defined as the sum of water from all other food items. Although we calculated magnesium intake from foods and drinks only, and not from

dietary supplements, no subjects used magnesium supplements and only 14 (0.4%) used multimineral supplements, rendering it unlikely that dietary supplementation had a major impact on the findings. Detailed descriptions of the methods used for calculating dietary intake and the validity of the DHQ have been published elsewhere (Sasaki *et al.*, 1998a, b, 2000). The Pearson correlation coefficient between DHQ and 3-day estimated dietary records was 0.48 for energy among 47 women (Sasaki *et al.*, 1998a). In addition, the Pearson correlation coefficients between DHQ and 16-day weighed dietary records were 0.69 for total dietary fiber, 0.62 for soluble dietary fiber, 0.70 for insoluble dietary fiber, 0.25 for total water, 0.25 for water from fluids, 0.64 for water from foods and 0.57 for magnesium in 92 women (S Sasaki, unpublished observations, 2006).

Constipation

A constipation questionnaire was developed based on a previous study (Garrigues *et al.*, 2004) and incorporated into the 20-page questionnaire for lifestyle during the previous 6 years. We used the definition of functional constipation recommended by an international workshop on the management of constipation (Rome I criteria) (Whitehead *et al.*, 1991). Although the Rome I criteria were modified in 1999 to the Rome II criteria (Thompson *et al.*, 1999), epidemiologic studies have consistently shown that the latter may be too restrictive for the diagnosis of constipation (Pare *et al.*, 2001; Garrigues *et al.*, 2004), and we therefore used the former. The following four questions were used to assess Rome I-defined functional constipation: (1) Do you strain during a bowel movement? (2) Do you feel an incomplete emptying sensation after a bowel movement? (3) How often are your stools hard? and (4) How many bowel movements do you usually have each week? These questions referred to the last 12 months. For questions 1–3, four answers were offered: never, sometimes ($<25\%$ of the time), often ($\geqslant 25\%$ of the time) and always. Functional constipation was defined as meeting two or more of the four criteria (an answer of *often* or *always* to questions 1–3 and <3 bowel movements per week (question 4)).

Other variables

In the questionnaires, subjects reported body weight and height, residential area, current smoking (yes or no) and oral medication usage (yes or no). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). We classified BMI into three categories (<18.5, 18.5–24.9 and $\geqslant 25\,kg/m^2$) according to the *Japan Society for the Study of Obesity* (Matsuzawa *et al.*, 2000). The reported residential areas were grouped into six categories (Hokkaido and Tohoku; Kanto; Hokuriku and Tokai; Kinki; Chugoku and Shikoku; and Kyushu) based on the regional blocks used in the National Nutrition Survey in Japan (Ministry of Health, Labour, and Welfare, 2004) (hereafter referred to as 'residential block'). The residential areas were also grouped into three categories according to population size (city with a population $\geqslant 1$ million; city with a population <1 million; and town and village) (hereafter referred to as 'size of residential area').

Additionally, subjects reported the time when they usually went to bed and arose in the morning, which was used to calculate sleeping hours, and the frequency and duration of high- and moderate-intensity activities, walking, and sedentary activities. For subjects whose recorded total hours were $<24\,h$, unrecorded hours were assumed to be spent on sedentary activities. For subjects whose recorded total hours were $>24\,h$, the total number of hours spent daily were proportionately decreased to equal 24. Each activities was assigned a metabolic equivalent (MET) value from a previously published table; 0.9 for sleeping, 1.5 for sedentary activity, 3.3 for walking, 5.0 for moderate-intensity activity and 7.0 for high-intensity activity (Ainsworth *et al.*, 1993, 2000). The number of hours spent per day on each activity was multiplied by the MET value of that activity, and all MET-hour products were summed to give a total MET-hour score for the day. This score essentially corresponds to the number of kilojoules per kilogram of body weight expended by an individual during the day. The standard value of basal metabolic rate for Japanese people is also expressed as the number of kilojoules per kilogram of body weight expended by an individual during the day. Physical activity level was then calculated by dividing total MET-hour score (kJ/kg of body weight/day) by the standard value of basal metabolic rate for Japanese women aged 18–29 years (99 kJ/kg of body weight/day) (Ministry of Health, Labour, and Welfare, 2005).

Statistical analysis

Associations between functional constipation (the dependent variable) and energy-adjusted (/4186 kJ) intakes of total, soluble and insoluble dietary fiber, total water, water from fluids, and water from foods and magnesium were examined. We calculated both crude and multivariate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for functional constipation for each quintile category of dietary variable using logistic regression analysis. Multivariate adjusted ORs were calculated by adjusting for BMI (three categories), residential block (six categories), size of residential area (three categories), current smoking (two categories), current alcohol drinking (two categories (yes or no) because of extremely low alcohol intake: mean $=0.8$ g/day), oral medication usage (two categories), physical activity level (quintiles) and energy intake (quintiles). We further conducted multivariate analyses including dietary fiber, water and magnesium simultaneously in order to investigate the independent associations with constipation. As results for the crude and multivariate analyses were similar for all variables analyzed, we present here only those derived from the multivariate models. Trend of association was assessed by a logistic regression model assigning scores

to the levels of the independent variable. All statistical analyses were performed using SAS statistical software, version 8.2 (SAS Institute Inc., Cary, NC, USA). All reported *P*-values are two-tailed, and a *P*-value of <0.05 was considered statistically significant.

Results

Basic characteristics of the subjects are shown in Table 1. Mean total dietary fiber intake was 6.4 g/4186 kJ, mean total water intake was 1025 g/4186 kJ and mean magnesium intake was 119 mg/4186 kJ. A total of 1002 women (26.2%) were classified as having constipation. Table 2 shows the multivariate adjusted ORs for constipation by quintiles of dietary variables. Dietary fiber intake was not associated with constipation. Further, no association was seen for total water intake or intake of water from fluids. However, low intake of water from foods was associated with increasing prevalence of constipation. In comparison with women in the first (lowest) quintile of intake of water from foods, the multivariate adjusted OR (95% CI) for women in the second, third, fourth and fifth quintiles were 0.72 (0.57, 0.90), 0.78 (0.62, 0.98), 0.71 (0.56, 0.89) and 0.77 (0.61, 0.97), respectively (*P* for trend = 0.04). Low magnesium intake was also associated with increasing prevalence of constipation. Compared with women in the first quintile of magnesium intake, the multivariate adjusted OR (95% CI) for women in the second, third, fourth and fifth quintiles were 0.70 (0.56, 0.88), 0.75 (0.60, 0.95), 0.73 (0.58, 0.92) and 0.79 (0.63, 0.996), respectively (*P* for trend = 0.09). Including dietary fiber, water and magnesium intake simultaneously in the models generally attenuated the association between dietary intake and constipation (see multivariate and nutrient-adjusted ORs in Table 2). However, these analyses did not materially change the relations of intake of water from foods and intake of magnesium to constipation, suggesting that both are independently associated with an increasing prevalence of constipation.

Table 1 Characteristics of 3825 Japanese women aged 18–20 years

	Mean ± s.d. or %
Age (years)	18.1 ± 0.3
Body height (cm)	157.9 ± 5.3
Body weight (kg)	52.3 ± 7.7
Body mass index (kg/m^2)	21.0 ± 2.8
<18.5	14.6
18.5–24.9	77.8
⩾25	7.6
Residential block	
Hokkaido and Tohoku	9.8
Kanto	34.3
Hokuriku and Tokai	14.0
Kinki	20.0
Chugoku and Shikoku	11.0
Kyushu	10.9
Size of residential area	
City with a population ⩾1 million	19.5
City with a population <1 million	65.2
Town and village	15.3
Current smoker	1.5
Current alcohol drinker	19.0
Oral medication user	9.9
Physical activity level	1.45 ± 0.15
Dietary intake	
Total energy (kJ/day)	7615 ± 2101
Total dietary fiber (g/4186 kJ)	6.4 ± 2.0
Soluble dietary fiber (g/4186 kJ)	1.7 ± 0.6
Insoluble dietary fiber (g/4186 kJ)	4.7 ± 1.5
Total water (g/4186 kJ)	1028 ± 360
Water from fluids (g/4186 kJ)	654 ± 337
Water from foods (g/4186 kJ)	374 ± 65
Magnesium (mg/4186 kJ)	118 ± 29
Functional constipation[a]	
No	73.8
Yes	26.2

[a]Defined according to the Rome I criteria (Whitehead *et al.*, 1991).

Discussion

To our knowledge, this study is the first to examine dietary fiber, water and magnesium intake in relation to Rome I-defined functional constipation. After controlling for a series of potential confounding factors, we found that a low intake of water from foods and magnesium was associated with an increasing prevalence of functional constipation. In contrast, no association was seen for dietary fiber, total water and water from fluids.

The prevalence of Rome I-defined functional constipation in the present group was 26.2%. A similar prevalence by these criteria has been reported in Canadian (21.0%) (Pare *et al.*, 2001) and Spanish (28.6%) (Garrigues *et al.*, 2004) women, whereas a somewhat smaller ratio was seen in elderly Singaporean women (10.5%) (Wong *et al.*, 1999).

Increased intake of dietary fiber is widely considered to protect against constipation, and several studies have indeed found an inverse relation between dietary fiber intake and constipation (Dukas *et al.*, 2003; Sanjoaquin *et al.*, 2004). Here, however, in common with several other studies (Campbell *et al.*, 1993; Towers *et al.*, 1994; Murakami *et al.*, 2006), we failed to find such an association. A possible explanation for this is that the dietary fiber intake of most subjects was too low to have a protective effect. Estimated intake in the present study (mean = 11.8 g/day) was, however, comparable to that observed in women aged 18–29 years in the Japanese National Nutrition Survey (mean-12.0 g/day) (Ministry of Health, Labour, and Welfare, 2004).

Although we saw no relation between the intake of total water and water from fluids, and constipation, a low intake of water from foods was associated with an increasing prevalence of constipation. To our knowledge, no previous observational study has investigated the relationship