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Research and Professional Briefs

Neighborhood Socioeconomic Disadvantage Is Associated with Higher Ratio of 24-Hour Urinary Sodium to Potassium in Young Japanese Women

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ABSTRACT

Information on the relationship of neighborhood characteristics to objective indicators of dietary intake is extremely limited. The aim of this observational cross-sectional study was to examine the association between neighborhood socioeconomic status (SES) and 24-hour urinary excretion of sodium and potassium in a population with a high ratio of urinary sodium to potassium. Subjects were 1,032 female Japanese dietetics students aged 18 to 22 years, residing in 293 municipalities in Japan. Neighborhood SES index was defined by seven municipal-level variables, namely unemployment, household overcrowding, poverty, education, income, home ownership, and vulnerable groups, with an increasing index signifying increasing neighborhood socioeconomic disadvantage. Urinary excretion of sodium and potassium was estimated from a single 24-hour urine sample. Neighborhood SES index was not significantly associated with 24-hour urinary excretion of sodium (mean value for each quartile of neighborhood SES: 133.5, 135.2, 126.5, and 141.7 mmol/day, respectively; *P* for trend 0.10) or potassium (mean value for each quartile: 43.5, 42.2, 38.4, and 42.5 mmol/day, respectively; *P* for trend 0.44). However, neighborhood SES index was significantly positively associated with the ratio of 24-hour urinary sodium to potassium (mean value for each quartile: 3.14, 3.28, 3.37, and 3.41, respectively; *P* for trend 0.03). This significant

association remained after adjustment for household SES variables (mean value for each quartile: 3.15, 3.35, 3.29, and 3.41, respectively; *P* for trend 0.04). Neighborhood socioeconomic disadvantage was associated with higher ratio of 24-hour urinary sodium to potassium in young Japanese women.

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Living conditions are shaped by characteristics of the residential environment; therefore, neighborhood characteristics may have some influence on lifestyle factors such as dietary habits, beyond any effects of the characteristics of an individual. In fact, a limited number of studies have shown that living in a socioeconomically disadvantaged neighborhood is associated with unfavorable dietary intake patterns (1-4). To our knowledge, all previous studies on this topic have used self-reported information on diet (1-3,5), with the exception of one article that used objective biomarkers (serum levels of carotenoids) (4). Evaluating dietary intake by use of biomarkers may serve as a way to bypass biases that are associated with self-reported dietary information.

We examined the association of a neighborhood socioeconomic status (SES) index, recently formulated for Japanese conditions (6), with 24-hour urinary excretion of sodium and potassium, an established biomarker of sodium and potassium intake (7-9), in a group of young Japanese women. High sodium and low potassium intakes are established determinants of several chronic illnesses such as hypertension and cardiovascular disease (10,11). According to the INTERSALT Study (12), Japanese are characterized by high urinary excretion of sodium and low urinary excretion of potassium, and hence a high ratio of urinary sodium to potassium. According to the National Health and Nutrition Survey in Japan, the major contributor of dietary sodium was seasonings (67%), whereas the top contributor of dietary potassium was vegetables (28%), followed by fish (10%) and fruit (9%) (13). A clear understanding of the relationship of neighborhood SES with urinary sodium and potassium in Japanese is thus of public health importance. In this study, we hypothesized that neighborhood socioeconomic disadvantage is associated with unfavorable profiles of urinary excretion of sodium and potassium.

METHODS

This observational cross-sectional study was conducted from February to March 2006 and from January to March

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2007 among female Japanese dietetic students from 15 institutions in Japan (N=1,176). A detailed description of the study design and survey procedure has been published elsewhere (14-18). Written informed consent was obtained from each participant, and also from a parent for participants aged <20 years. The study protocol was approved by the Ethics Committee of the National Institute of Health and Nutrition, Japan.

In Japan, the total of 2,372 municipalities consisted of the 164 wards, 736 cities, 1,178 towns, and 294 villages (as of October 1, 2005) (19). We used municipalities as proxies for neighborhoods (5), which is in accordance with previous Western studies where some administrative divisions are used as proxies for neighborhoods (1-4). Study participants were linked to their municipalities using their home address.

In total, 1,105 women undertook 24-hour urine collection. For analysis, we selected women aged 18 to 22 years (n=1,083). We then excluded women with missing information on the variables used (n=18). We further excluded those whose 24-hour urine collection was considered incomplete (n=35), as assessed using information on urinary creatinine excretion and body weight based on a strategy proposed by Knuiman and colleagues (20); this creatinine-based strategy has been validated against the para-aminobenzoic acid check method in a subsample of the present participants (18,21). The final analysis sample comprised 1,032 women, who resided in 293 different municipalities in Japan.

We constructed a neighborhood SES index at the municipality level (5) using seven variables determined by a factor analysis (6). These variables were unemployment (percentage of unemployed persons aged ≥ 15 years), household overcrowding (average floor space per residential dwelling), poverty (number of households receiving public assistance per 1,000 households), education (percentage of persons aged 20 to 64 years who had completed college or university), income (total taxable income divided by total population), home ownership (percentage of owned houses to total residential households), and vulnerable groups (percentage of households of single persons aged ≥ 65 years to total households) (6). Data were derived from the 2005 census (19) and other government surveys (19,22,23). These seven variables were combined into a neighborhood summary score (ie, neighborhood SES index) constructed by summing *z* scores for each of the seven variables (for unemployment, poverty, income, and vulnerable groups, data were log transformed before calculating *z* scores; *z* scores for household overcrowding, education, income, and home ownership were multiplied by -1 before summing), with a higher neighborhood SES index signifying increasing neighborhood socioeconomic disadvantage (6). A detailed description of the neighborhood SES index has been published elsewhere (5).

A single 24-hour urine sample was collected from each participant. A detailed description of the procedure of 24-hour urine collection and the biochemical measurements has been published elsewhere (17,18). Briefly, subjects were asked to collect all urine specimens during a 24-hour period, and to record the time of the start and end of the collection period and the estimated volume of all missing urine specimens. We adjusted 24-hour urine vol-

ume by self-reported collection time (calculated from the self-reported time of the start and end of the collection period) and missing urine volume; this adjustment strategy has been validated using the para-aminobenzoic acid check method in a subsample of the present participants (18,21).

All urine samples taken during the 24-hour period were carefully mixed, and several aliquots were taken and transported at -20°C to a laboratory (SRL Inc, Tokyo, Japan, in 2006 and Mitsubishi Kagaku Bio-Clinical Laboratories Inc, Tokyo, Japan, in 2007). According to the standard procedure at each laboratory, sodium and potassium concentrations were measured using the ion-selective electrode method (24), and creatinine using the enzymatic assay method (25). Total 24-hour excretion was calculated by multiplying the measured concentration by the volume of 24-hour urine. Estimates of urinary sodium and potassium and the ratio of urinary sodium to potassium were log-transformed.

All statistical analyses were performed using SAS statistical software (version 9.1, 2003, SAS Institute Inc, Cary, NC). Using the PROC GLM procedure, linear regression models were constructed to examine the association of neighborhood SES index with urinary excretion of sodium and potassium and the ratio of urinary sodium to potassium. For analyses, participants were categorized into quartiles according to neighborhood SES index. Multivariate-adjusted mean values (with 95% confidence intervals) of urinary markers were calculated by quartile of neighborhood SES index. First, the association was examined with adjustment for survey year (14-16,21). Then, household SES variables such as institution type (5,26) and living status (5,27) were further included. Finally, geographical variables such as region (5) and municipality level (5) were further included, considering regional or urban-rural differences in neighborhood SES in Japan (6,28,29), although this may be an overadjustment. We tested for linear trends with increasing levels of neighborhood SES index by assigning each participant the 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. As the great majority of municipalities had only a few study participants (median=1; interquartile range=1, 3), no special methods were needed to account for within-neighborhood correlations in outcomes (30).

RESULTS AND DISCUSSION

Mean \pm standard deviation of physical characteristics was as follows: 19.6 ± 1.0 years for age, 158.4 ± 5.4 cm for height, 53.3 ± 7.3 kg for weight, and 21.2 ± 2.5 for body mass index. Median (interquartile range) of urinary excretion of sodium and potassium was 138.6 (106.0, 176.4) and 41.9 (33.2, 52.4) mmol/day, respectively. Median (interquartile range) of the ratio of urinary sodium to potassium was 3.24 (2.46, 4.24).

As shown in Table 1, neighborhood SES index was associated with each of the seven neighborhood SES variables in the expected direction. Neighborhood SES index was also associated with region, municipality level, and institution type. In the higher quartiles of neighborhood SES index (increasing neighborhood socioeconomic disadvantage), there were more participants living in Hok-

Characteristic	All (N=1,032)		Quartile 1 (n=255)		Quartile 2 (n=269)		Quartile 3 (n=243)		Quartile 4 (n=265)		P value ^b
	Mean	95% CI ^a	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Neighborhood SES index											
Median	-0.07		-3.15		-0.97		0.20		3.78		—
Range	-12.13, 27.33		-12.13, -2.02		-2.01, -0.07		-0.06, 1.29		1.30, 27.33		—
Percentage of unemployed persons aged ≥15 y ^c	6.0	5.9, 6.0	5.2	5.1, 5.3	5.6	5.5, 5.6	6.4	6.3, 6.5	6.8	6.7, 7.0	<0.0001
Average floor space per residential dwelling (m ²)	89.2	88.0, 90.4	95.8	93.2, 98.4	89.3	87.3, 91.3	88.5	86.4, 90.6	83.4	81.0, 85.8	<0.0001
No. of households receiving public assistance (/1,000 households) ^c	13.9	13.5, 14.4	8.6	8.2, 9.0	14.6	14.2, 15.1	11.6	11.0, 12.2	24.5	23.2, 25.9	<0.0001
Percentage of persons completing college or university aged 20 to 64 y	33.1	32.7, 33.6	35.5	34.4, 36.6	34.5	33.6, 35.5	31.6	30.9, 32.3	30.8	30.0, 31.6	<0.0001
Total taxable income divided by total population (thousand yen) ^c	1,455	1,437, 1,473	1,570	1,525, 1,617	1,528	1,500, 1,557	1,416	1,389, 1,444	1,318	1,287, 1,350	<0.0001
Percentage of owned houses to total residential households	62.2	61.5, 62.9	68.1	66.6, 69.5	63.2	62.2, 64.3	62.3	61.3, 63.3	55.4	53.9, 56.9	<0.0001
Percentage of households of single persons aged ≥65 y to total households ^c	6.5	6.4, 6.6	5.2	5.1, 5.4	6.3	6.1, 6.4	6.4	6.2, 6.5	8.4	8.2, 8.6	<0.0001
Survey year (%)											
2006	39.0		39.2		33.1		44.9		39.3		0.39
2007	61.1		60.8		66.9		55.1		60.8		<0.0001
Region (%)											
Hokkaido and Tohoku	2.7		0		0.7		0.4		9.4		
Kanto	55.1		69.4		64.7		63.0		24.5		
Hokuriku and Tokai	13.3		9.0		24.5		17.7		1.9		
Kinki	11.0		17.3		7.4		1.7		17.0		
Chugoku and Shikoku	3.6		0.8		0.7		0		12.5		
Kyushu	14.3		3.5		1.9		17.3		34.7		
Municipality level (%)											
Ward	15.7		7.8		9.3		7.8		37.0		
City	78.7		78.4		89.6		87.2		60.0		
Town and village	5.6		13.7		1.1		4.9		3.0		
Institution type (%)											
4-year private	69.3		69.0		57.6		76.5		74.7		
2-year private	4.9		2.8		1.1		14.8		1.9		
4-year public	13.5		18.0		8.2		6.6		20.8		
2-year public	12.3		10.2		33.1		2.1		2.6		
Living status (%)											
Living with family	60.3		66.2		63.9		37.0		70.2		0.21
Living alone	35.7		28.6		30.9		59.3		25.7		
Living with others	4.1		3.1		5.2		3.7		4.2		

^aCI=confidence interval.

^bFor continuous variables, a linear trend test was used with the median value in each quintile category as a continuous variable in linear regression; for categorical variables, a Mantel-Haenszel χ^2 test was used.

^cCalculated using back transformation of natural-log transformed values.

Table 2. Urinary excretion of sodium and potassium and the ratio of urinary sodium to potassium of 1,032 Japanese women aged 18 to 22 years according to quartile category of neighborhood socioeconomic status index

Characteristic	Quartile 1 (n=255)		Quartile 2 (n=269)		Quartile 3 (n=243)		Quartile 4 (n=265)		P value ^b
	Mean	95% CI ^a	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Urinary excretion of sodium (mmol/d)^c									
Model 1 ^d	133.5	127.1, 140.1	135.2	128.9, 141.8	126.5	120.3, 133.0	141.7	135.1, 148.6	0.10
Model 2 ^e	132.2	125.8, 138.8	134.5	127.9, 141.4	130.5	123.6, 137.8	139.7	133.0, 146.8	0.12
Model 3 ^f	130.9	124.1, 138.0	133.8	126.9, 141.1	130.8	123.6, 138.3	141.5	133.2, 150.3	0.10
Urinary excretion of potassium (mmol/d)^c									
Model 1 ^d	43.5	41.7, 45.5	42.2	40.5, 44.0	38.4	36.7, 40.1	42.5	40.7, 44.4	0.44
Model 2 ^e	43.0	41.2, 44.9	41.2	39.4, 43.1	40.6	38.7, 42.6	41.9	40.1, 43.7	0.48
Model 3 ^f	43.1	41.2, 45.2	40.7	38.9, 42.7	40.5	38.6, 42.6	42.3	40.1, 44.6	0.58
Ratio of urinary sodium to potassium (mmol:mmol)^c									
Model 1 ^d	3.14	2.98, 3.30	3.28	3.12, 3.45	3.37	3.20, 3.55	3.41	3.24, 3.58	0.03
Model 2 ^e	3.15	2.98, 3.31	3.35	3.17, 3.53	3.29	3.10, 3.48	3.41	3.24, 3.59	0.04
Model 3 ^f	3.11	2.94, 3.29	3.37	3.19, 3.56	3.30	3.11, 3.50	3.41	3.20, 3.63	0.06

^aCI=confidence interval.

^bA linear trend test was used with the median value in each quintile category as a continuous variable in linear regression.

^cCalculated using back transformation of natural-log transformed values.

^dAdjusted for survey year (2006 and 2007).

^eAdjusted for survey year, institution type (4-y private, 2-y private, 4-y public, and 2-y public), and living status (living with family, living alone, and others).

^fAdjusted for survey year, institution type, living status, region (Hokkaido and Tohoku, Kanto, Hokuriku and Tokai, Kinki, Chugoku and Shikoku, and Kyushu), and municipality level (ward, city, and town and village).

kaido and Tohoku, Chugoku and Shikoku, and Kyushu, and fewer living in Kanto, Hokuriku and Tokai, and Kinki; more participants living in wards and fewer living in towns and villages; and more participants attending 4-year private, 2-year private, and 4-year public institutions and fewer attending 2-year public institutions.

As shown in Table 2, neighborhood SES index was not significantly associated with estimates of 24-hour urinary excretion of sodium or potassium. A similar relation was observed when creatinine-adjusted values were used (data not shown). In contrast, higher neighborhood SES index (increasing neighborhood socioeconomic disadvantage) was significantly associated with higher ratio of 24-hour urinary sodium to potassium. This significant positive association remained after adjustment for household SES variables. However, the association disappeared after further adjustment for geographical variables.

Only one previous study has examined the association of neighborhood with objective markers of dietary intake (4). In that study of US adults, neighborhood deprivation was associated with lower serum levels of carotenoids (4). Our findings are consistent with the US study in terms of the observation that disadvantaged neighborhoods are associated with unfavorable aspects of dietary intake.

The reason for the association between neighborhood SES and the ratio of urinary sodium to potassium is not known. Neighborhoods may influence dietary intake (urinary excretion) of sodium and potassium through characteristics of the area itself (3), such as the availability of health foods (31,32). Neighborhoods may also influence dietary intake of sodium and potassium through the role of psychosocial factors (3), given a suggestion that peo-

ple's behavior is influenced by the norms and values of those around them (33). In addition, the association between neighborhood SES and the ratio of urinary sodium to potassium may merely reflect geographical difference in the urinary sodium to potassium ratio, given that whereas neighborhood SES was associated with region and municipality level, the association between neighborhood SES and the ratio of urinary sodium to potassium disappeared after adjustment for these geographical variables. It is also possible that neighborhood SES contributes to regional and urban-rural differences in the ratio of urinary sodium to potassium, and that geographical variables are a proxy for unmeasured neighborhood-level factors that covary with those we are investigating. The reason for the lack of association between neighborhood SES and urinary sodium and potassium, when assessed individually, is also unknown; neighborhood SES might have stronger influence on some aspects of dietary patterns (eg, combination of nutrients) rather than on intake of each individual nutrient.

Several limitations of the present study deserve mention. First, our results of selected dietetics students are not likely to extrapolate to the general population. The participants likely had more nutrition knowledge than an average person and were not necessarily socioeconomically disadvantaged (at least at the individual level). Given the findings of an association in this population, in which nutrition knowledge would affect what they consume regardless of neighborhood, associations between neighborhood SES and diet might be expected to be stronger in the general population. Second, we relied on census-based measures at the municipality level as proxies for neighborhoods, but these might not correspond to socially defined neighborhoods. In addition, municipality

in Japan may be a somewhat large unit of neighborhoods, given that the median population of 293 municipalities was 124,393 (interquartile range 92,210 to 274,481). Our study is also limited by the use of the neighborhood SES score as an indirect proxy for the specific features of neighborhoods that may be more relevant (30). Third, urinary excretion of sodium and potassium was estimated based on a single 24-hour urine sample, which is not optimal for characterizing individual habitual dietary intake and introduces random errors (34). Fourth, we cannot rule out residual confounding. In particular, parents' SES variables were not available, although these may be at least partly reflected by household SES variables. Fifth, the short duration of the data collection period may lead to an under-representation of the typical potassium and sodium excretion of the study population. Finally, the cross-sectional nature of the study hampers the drawing of any conclusions on causal inferences between neighborhood SES and 24-hour urinary excretion of sodium and potassium.

CONCLUSIONS

Neighborhood socioeconomic disadvantage was associated with a higher ratio of 24-hour urinary sodium to potassium in a group of young Japanese women. This finding suggests that the socioeconomic characteristics of neighborhoods influence dietary intake and hence health variables. Efforts to reduce inequalities in neighborhood SES may represent an important strategy in improving the health status of individuals.

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Original Article

Association of *trans* fatty acid intake with metabolic risk factors among free-living young Japanese women

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Objective: We examined cross-sectional associations of total, hydrogenated, and natural *trans* fatty acid intake with selected metabolic risk factors in young Japanese women. **Methods:** Subjects were 1136 Japanese female dietetic students aged 18-22 years. Dietary intake was estimated using a validated, self-administered diet history questionnaire. Associations between *trans* fatty acid intake and metabolic risk factors were examined with multivariate linear regression analysis, with control for potential covariates. Dietary covariates included intake of energy, total fat, and saturated fatty acids (model 1); monounsaturated fatty acids instead of saturated fatty acids (model 2); and polyunsaturated fatty acids instead of saturated fatty acids (model 3). **Results:** Mean (standard deviation) total *trans* fatty acid intake was 0.90% (0.30%) of total energy. Hydrogenated *trans* fatty acids contributed 77% of total *trans* fatty acid intake. Total *trans* fatty acid intake was significantly and positively associated with waist circumference, triacylglycerol, and glycated hemoglobin, except in the analysis of triacylglycerol with adjustment for monounsaturated fatty acids. No associations were found between total *trans* fatty acid intake and body mass index, cholesterol, or glucose. Hydrogenated *trans* fatty acid intake was significantly and positively associated only with waist circumference and glycated hemoglobin. No association was observed for natural *trans* fatty acid intake. **Conclusion:** hydrogenated *trans* fatty acid intake was positively associated with several metabolic risk factors among free-living young Japanese women with relatively low intake.

Key Words: *trans* fatty acid intake, metabolic risk factors, young Japanese women, cross-sectional study, Asian population

INTRODUCTION

Trans fatty acids, unsaturated fatty acids formed during the partial hydrogenation of commercial liquid vegetable oils to semi-solid fats, are found in margarine, shortening, and frying fats. A summary of human intervention trials has suggested that diets high in *trans* fatty acids have significantly adverse effects on blood lipid concentrations;¹ and *trans* fatty acids may also have a negative effect on insulin sensitivity,² albeit that not all trials have found such an effect.^{3,4} Although these results suggest the possible association of *trans* fatty acid intake with several metabolic risk factors, intake levels were generally higher (3.8%-20% energy)¹⁻⁵ than those in free-living populations (0.87%-4.30% energy),⁶⁻¹³ hampering their direct extrapolation to free-living populations.

Few observational studies have investigated the association between *trans* fatty acid intake and metabolic risk factors among free-living populations, and results have been inconsistent. Significant unfavorable associations have been reported for blood lipid concentrations,^{9,10,12} glucose concentrations,¹² waist circumference,^{11,12} and body weight¹³ versus no significant association for blood lipid concentrations,^{7,10} glucose concentrations⁸ and body mass index (BMI).¹² Moreover, different effect on metabolic risk factors between hydrogenated *trans* fatty acids,

produced by partial hydrogenation of commercial liquid vegetable oils, and natural *trans* fatty acid, found naturally in ruminants as a result of bio-hydrogenation of polyunsaturated fatty acids, has been suggested.¹⁰

Despite the preventive value of early identification of modifiable dietary factors among healthy and young populations and the possible onset of chronic diseases associated with each of these metabolic risk factors in the young,¹⁴ most of these observational studies were conducted in middle-aged and elderly populations (mean subject age across studies: >30 years). Further, they were largely conducted in western populations,^{7-11,13} and information from Asian countries, including Japan, where dietary fat intake is relatively low,¹⁵ is limited.¹²

We conducted a cross-sectional study of the associations between total, hydrogenated, and natural *trans* fatty acid intake and metabolic risk factors, including BMI,

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waist circumference, and serum levels of cholesterol [total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL)], triacylglycerol, glucose, and glycated-hemoglobin (HbA1c) among free-living young Japanese women.

MATERIAL AND METHODS

Study population

The present study was based on a multi-center cross-sectional study conducted from February to March 2006 and from January to March 2007 among female dietetic students at 15 institutions in Japan. All measurements at each institution were carried out in accordance with the survey protocol. The survey protocol has been described in detail elsewhere.¹⁶⁻¹⁸ Briefly, staff at each institution provided an outline of the survey to potential subjects. Those who responded positively were provided detailed written and oral explanations of the survey's general purpose and procedure. A total of 1176 Japanese women took part. For the present analyses, those aged <18 or >22 years ($n = 22$) were excluded since the majority of Japanese dietetic students are in the 18-22 years of age range. We also excluded those who provided incomplete survey questionnaires ($n = 1$); reported extremely low or high energy intake (<500 or >4000 kcal/day; $n = 2$); were currently receiving dietary counseling from a doctor or dietitian ($n = 13$); had a history of a diagnosis of diabetes, hypertension, or cardiovascular disease ($n = 1$); or had no data for body height and weight ($n = 2$). Those who provided non-fasting blood samples ($n = 34$) or who had missing information on any metabolic risk factor ($n = 16$) were excluded from the analyses of biochemical measurements. Some participants fell into more than one exclusion category. A total of 1136 women were eligible for BMI and waist circumference analyses, and 1087 for serum cholesterol (total, LDL, and HDL), triacylglycerol, glucose, and HbA1c. The study was approved by the Ethics Committee of the National Institute of Health and Nutrition. Written informed consent was obtained from all subjects and from a parent of subjects aged <20 years. Basic physical characteristics of the subjects ($n = 1136$) have been published elsewhere.¹⁶⁻¹⁸

Dietary assessment

A previously validated, self-administered, comprehensive diet history questionnaire (DHQ)¹⁹⁻²² was used. The DHQ is a 16-page structured questionnaire about dietary habits during the previous month. Responses to the DHQ and to an accompanying lifestyle questionnaire were checked at least twice for completeness. The DHQ and lifestyle questionnaire have been described in detail elsewhere.¹⁹⁻²² Estimated dietary intake for a total of 150 food and beverage items, energy, and selected nutrients (except *trans* fatty acids) were calculated using an ad hoc computer algorithm for the DHQ, which was based on the Standard Tables of Food Composition in Japan.²³ Estimated total, hydrogenated, and natural *trans* fatty acid intake was calculated based on a food composition database developed for the present study.

To minimize the influence of dietary misreporting, a known problem of self-reported dietary assessment methods,^{24,25} nutrient intake was energy-adjusted using the

density method (i.e., percentage of energy for energy-yielding nutrients and amounts per 1000 kcal of energy for other nutrients).²⁶ The methods used to calculate dietary intake and the validity of the DHQ with commonly studied nutritional variables have been described in detail elsewhere.¹⁹⁻²² For example, the relative validity of dietary intake estimated from the DHQ was examined against that from 3-day weighed diet records in 47 women. Pearson correlation coefficients between the two methods were 0.55 for total fat, 0.75 for saturated fatty acids, 0.50 for monounsaturated fatty acids, and 0.37 for polyunsaturated fatty acids.¹⁹

Validation of *trans* fatty acid intake

The relative validity of *trans* fatty acid intake estimated from the DHQ was examined against that from the 16-day weighted diet records among 92 women 31-69 years of age. Subjects completed a 4-nonconsecutive-day (one weekend day and three weekdays) semi-weighed diet record (DRs) four times, at intervals of approximately 3 months (from November 2002 to September 2003). Details of the diet record procedure are provided elsewhere.²² Briefly, during the orientation session, registered dietitians gave the subjects both written and verbal instructions on how to keep the DRs, provided recording sheets and a digital scale, and asked the subjects to record and weigh all foods and beverages consumed on each recording day. All collected records were checked by trained registered dietitians in the respective local centers and then again in the study center. A total of 1320 food and beverage items appeared in the DR.

Since a comprehensive *trans* fatty acid food composition tables is not available in Japan, we developed a *trans* fatty acid database for 1976 foods appearing in the Standard Tables of Food Composition in Japan.²³ Among the 1976 foods, 1469 were determined to contain no *trans* fatty acids since they contained no or only a trace amount of fat²³ and no hydrogenated oils or ruminants.²⁷ Determination of the *trans* fatty acid content was done via a 4-step process, as follows:

Step 1: The primary data source for *trans* fatty acid values of the remaining 507 foods was direct chemical analysis.²⁸⁻⁴³ We searched PubMed, CiNii, and Medical Online Library databases for English or Japanese literature reporting analyses of *trans* fatty acid content of foods assessed by gas chromatography⁴⁴ conducted in Japan. We also included data on analytic *trans* fatty acid values from two unpublished reports (personal communication; T. Maruyama, 2001) and three foods provided in the Standard Tables of Food Composition in Japan (i.e., soft margarine, fat spread, and shortening).²³

Trans fatty acid values in the analytic data²⁸⁻⁴³ were converted to grams per 100 g of food, adjusting for total fat content in the Standard Tables of Food Composition in Japan²³ using the following equation: *Trans* fatty acid (g)/100 g of food = [*trans* fatty acid (g)/total fat (g) in reference] × [total fat (g)/100g of food in the Standard Tables of Food Composition in Japan]. When only one article existed and this article analyzed the *trans* fatty acid content in a single example of a food only, this value was selected ($n = 13$). When only one article existed and this article analyzed the *trans* fatty acid content of several

samples and reported minimum, maximum, and/or mean values, we selected the mean value for the food ($n = 71$). When multiple articles existed but reported different mean *trans* fatty acid values for a specific food, we calculated the mean value by weighting the number of foods analyzed in each article ($n = 59$).

Step 2: When the *trans* fatty acid value for a specific food (except meat cuts) could not be obtained using Step 1 but an analytic value was available for a similar food having similar nutrient composition²³ within the same food group by Step 1, that value (*trans* fatty acid % of total fat) was assigned ($n = 173$). When the analytic *trans* fatty acid value of a specific meat cut was unavailable (Step 1) but an analytic value for a similar part was ($n = 172$), that value was assigned.

Step 3: For food products where *trans* fatty acid values could not be determined using Steps 1 and 2, but for which the manufacturer had a presence in both Japan and the US, we compared the nutrient composition of the food in Japan, as shown on the website of the company, with that of the US, as provided in the ESHA Food Processor SQL (ESHA Research, Salem, Oregon), and assigned the value obtained from the ESHA ($n = 3$).

Step 4: When the *trans* fatty acid values for a specific food were unavailable using Steps 1-3, we then imputed values by referring to recipes⁴⁵ and the nutrient composition of foods²³ ($n = 16$). Among 16 foods, 4 foods were determined to contain *trans* fatty acids.

Using the *trans* fatty acid database created above ($n = 1976$), we matched food codes in the database and those in the DHQ. A total of 48 foods in the DHQ were determined to contain *trans* fatty acids; among them, *trans* fatty acid values for 37 foods were determined through direct matching of published data. *Trans* fatty acid values for the remaining 11 foods were determined as follows: direct matching from unpublished data ($n = 1$); direct matching from both published and unpublished data ($n = 2$); assignment of a similar food value whose *trans* fatty acid value was determined by direct matching from published data ($n = 3$); assignment of a similar food value whose *trans* fatty acid value was determined by direct matching from both published and unpublished data ($n = 1$); direct matching obtained from ESHA Food Processor ($n = 2$); and direct matching of ingredients in a recipe with published data ($n = 2$).

Intake of energy and fatty acids were estimated based on the estimated intakes of all items and the Standard Tables of Food Composition in Japan.²³ Total, hydrogenated, and natural *trans* fatty acid intake was estimated based on the database created in the present study. Pearson's correlation coefficients between the DHQ and 16-day weighed diet records were 0.58 for total fat, 0.67 for saturated fatty acids, 0.54 for monounsaturated fatty acids, 0.34 for polyunsaturated fatty acids, 0.63 for total *trans* fatty acids, 0.58 for hydrogenated *trans* fatty acids, and 0.39 for natural *trans* fatty acids (unpublished observations, S. Sasaki, 2008).

Metabolic risk factors

Metabolic risk factors were assessed 1-3 days after completion of the questionnaires. The clinical measurements used have been described in detail elsewhere.¹⁷ Briefly,

body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index was calculated as body weight (kg) divided by the square of body height (m²). Waist circumference was measured at the level of the umbilicus to the nearest 0.1 cm. Overnight fasting blood samples were collected with vacuum tubes which contained no preservative, allowed to clot, and centrifuged at 3000 g for 10 minutes at room temperature to separate the serum. Blood samples for HbA1c were also collected in evacuated tubes containing no additives. To avoid significant degradation, blood samples were transported by car or airplane at -20 °C to a laboratory in Tokyo, Japan (SRL Inc in 2006 and Mitsubishi Kagaku Bio-Clinical Laboratories Inc in 2007) and assayed within 1-2 days of collection. Serum concentrations of total, LDL, and HDL cholesterol, triacylglycerol, and glucose were measured by enzymatic assay methods. Glycated hemoglobin was measured by latex agglutination-turbidimetric immunoassay. In-house quality-control procedures for all assays were conducted at the respective laboratory.

Other variables

The DHQ included self-reported current alcohol drinking (yes or no) and rate of eating (slow, medium, or fast).¹⁶ The lifestyle questionnaire included self-reported residential area, which was grouped into one of three geographical regions [north (Kanto, Hokkaido, and Tohoku); central (Tokai, Hokuriku, and Kinki); and south (Kyushu and Chugoku); hereafter referred to as 'residential block'].¹⁶ The residential areas were also grouped into three categories according to population size (city with population ≥ 1 million; city with population < 1 million; and town and village; hereafter referred to as 'size of residential area'). Current smoking (yes or no) was reported in the lifestyle questionnaire.¹⁶ Physical activity (total metabolic equivalents-h/day) was calculated as the average metabolic equivalent-hours per day⁴⁶ 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.¹⁶

Statistical analyses

All statistical analyses were performed using SAS statistical software (version 9.1; SAS Institute Inc, Cary, NC). Linear regression models were used (in PROC GLM) to assess the associations of total, hydrogenated, and natural *trans* fatty acid intake with eight metabolic risk factors. For the analyses, subjects were divided into quintiles according to total, hydrogenated, and natural *trans* fatty acid intake. The mean \pm standard error for metabolic risk factor values were calculated by quintiles of total, hydrogenated, and natural *trans* fatty acid intake after multivariate adjustments for potential confounding variables. Covariates included residential block, size of residential area, survey year (2006 or 2007; to account for the different laboratories used for the 2006 and 2007 surveys, notwithstanding that they used the same assay methods), current smoking, current alcohol drinking, rate of eating, physical activity (continuous), BMI (continuous; except for the analysis of BMI itself), waist circumference (con-

tinuous; except for the analysis of waist circumference itself), and intake of energy (continuous), total fat (continuous), and saturated fatty acids (continuous; model 1). Monounsaturated fatty acids (continuous; model 2) or polyunsaturated fatty acids (continuous; model 3) were added in place of saturated fatty acids. Linear trends with increasing levels of total, hydrogenated, and natural *trans* fatty acid intake were tested by assigning each subject the median value for the category and modeling this value as a continuous variable. All reported *p* values are two-tailed, and a *p* value of < 0.05 was considered statistically significant.

RESULTS

Mean (standard deviation) dietary intake of all subjects (*n* = 1136; those included in the analyses of BMI and waist circumference) is shown in Table 1. Intake of total, hydrogenated, and natural *trans* fatty acid intake ranged from 0.26% to 2.25%; 0.09 to 2.18%; and 0.01 to 0.64% of energy, respectively. Hydrogenated *trans* fatty acids contributed to 77% of total *trans* fatty acid intake. Major food groups contributing to total *trans* fatty acid intake were fat and oil (24.3%), bakery (20.2%), and confections (19.5%). Mean BMI and waist circumference were 21.3 (2.7) kg/m² and 72.9 (7.1) cm, respectively.

Potential confounding factors for all subjects (*n* = 1136) according to quintile of total *trans* fatty acid intake are shown in Table 2. Women in higher quintiles of intake were more likely to live in the north region and to have higher intake of energy, total fat, and saturated,

monounsaturated, and polyunsaturated fatty acids. By quintile of total *trans* fatty acid intake, results were similar for potential confounding factors and dietary intake among subjects included in the analyses of serum cholesterol (total, LDL, and HDL), triacylglycerol, glucose, and Hb A_{1c} (*n* = 1087; data not shown). The results were similar according to quintile of hydrogenated and natural *trans* fatty acid intake for all subjects (*n* = 1136) or among subjects included in the analyses of serum cholesterol (total, LDL, and HDL), triacylglycerol, glucose, and Hb A_{1c} (*n* = 1087), except for polyunsaturated fatty acid intake in the analysis by quintile of natural *trans* fatty acid intake (*p* = 0.06) (data not shown).

The multivariate-adjusted mean values for metabolic risk factors across quintiles of total *trans* fatty acid intake are shown in Table 3, 4, and 5, respectively. After adjustment for potential confounding factors, total *trans* fatty acid intake was positively associated with waist circumference, triacylglycerol, and HbA_{1c} (*p* for trend ≤ 0.046; models 1-3), except for the analysis of triacylglycerol with adjustment for monounsaturated fatty acids (model 2). No associations were found for total *trans* fatty acid intake with other metabolic risk factors. Additionally, hydrogenated *trans* fatty acid intake was independently positively associated with waist circumference and HbA_{1c} (*p* for trend ≤ 0.03; models 1-3), but not with other metabolic risk factors (Table 4). Conversely, natural *trans* fatty acid was not associated with any of the metabolic risk factors (Table 5).

Table 1. Dietary intake of subjects (*n* = 1,136)[†]

Variable	
Energy intake (kcal/day)	1752 ± 447
Nutrient intake	
<i>Trans</i> fatty acids (% energy)	0.90 ± 0.30
Hydrogenated <i>trans</i> fatty acids (% energy)	0.71 ± 0.29
Natural <i>trans</i> fatty acids (% energy)	0.19 ± 0.08
Total fat (% energy)	29.3 ± 5.2
Saturated fatty acids (% energy)	8.5 ± 2.0
Monounsaturated fatty acids (% energy)	10.2 ± 2.2
Polyunsaturated fatty acids (% energy)	6.4 ± 1.3
Contributions of food groups to <i>trans</i> fatty acid intake (%) [‡]	
Fat and oil	24.3
Bakery	20.2
Confections	19.5
Milk and milk products	13.4
Meat and meat products	8.7
Soft margarine	5.1
French fries	3.6
Others	5.2

[†] Values are mean ± standard deviation or contribution (%)

^{‡‡} *Trans* fatty acid value for food groups were determined by directly matching *trans* fatty acid value in published data with several exceptions indicated in parenthesis below; (1) direct matching from unpublished data (*n* = 1); (2) direct matching from both published and unpublished data (*n* = 2); (3) assignment of a similar food value whose *trans* fatty acid value was determined by direct matching from published assessment data (*n* = 3); (4) assignment of a similar food value whose *trans* fatty acid value was determined by direct matching from both published and unpublished data (*n* = 1); (5) direct matching obtained from ESHA Food Processor (*n* = 2); and (6) direct matching of ingredients in a recipe with published data (*n* = 2).

[§] Food groups were defined as follows: fat and oil [i.e., butter, refined vegetable oil, salad dressing (6), and mayonnaise]; bakery [i.e., white bread (2), butter roll (2), danish and pastry, croissant (1), pancake (3), pizza (5), and Japanese-style pancake (4)]; confections [i.e., potato chips, Japanese-style rice crackers, snacks, cakes, cookies, chocolate, doughnuts, energy bar (3), traditional Japanese azuki paste (6), traditional Japanese sweets, high-fat ice cream, low-fat ice cream, and regular ice cream]; milk and milk products [i.e., whole milk, low-fat milk, skim milk, sugar-added yogurt, nosugar-added yogurt, and sugar and nosugar-added yogurt, process cheese, cottage cheese, and coffee cream]; meat and meat products (i.e., ground meat, chicken, poultry, beef, liver meat, ham and sausages, and bacon); soft margarine; French fries; and others [i.e., instant ramen noodles, instant Chinese soup, instant corn soup (3), cornflake (5), tofu products, and retort foods].

Table 2. Selected characteristics and dietary intake according to quintile (Q) of total *trans* fatty acid intake ($n = 1136$)[†]

	Q1 ($n = 227$)	Q2 ($n = 227$)	Q3 ($n = 228$)	Q4 ($n = 227$)	Q5 ($n = 227$)	P^{\ddagger}
Median (range) value of total <i>trans</i> fatty acid intake (% energy)	0.56 (0.26-0.65)	0.72 (0.66-0.78)	0.88 (0.79-0.94)	1.03 (0.95-1.13)	1.32 (1.14-2.25)	
Residential block						
North (Kanto, Hokkaido, and Tohoku)	109 (48)	125 (56)	120 (53)	145 (64)	137 (60)	0.004
Central (Tokai, Hokuriku, and Kinki)	66 (29)	47 (20)	56 (24)	55 (24)	51 (23)	
South (Kyushu and Chugoku)	52 (23)	55 (24)	52 (23)	27 (12)	39 (17)	
Size of residential area						
City with population ≥ 1 million	27 (12)	37 (16)	42 (18)	33 (15)	44 (19)	0.14
City with population < 1 million	183 (81)	170 (75)	178 (79)	180 (79)	172 (76)	
Town and village	17 (7)	20 (9)	8 (3)	4 (6)	11 (5)	
Survey year						
2006	82 (36)	98 (43)	98 (43)	85 (37)	98 (43)	0.33
2007	145 (64)	129 (57)	130 (57)	142 (63)	129 (57)	
Current smoker	8 (4)	7 (3)	4 (2)	3 (1)	5 (3)	0.50
Current alcohol drinker	89 (39)	108 (48)	103 (45)	98 (43)	87 (38)	0.22
Rate of eating						
Slow	75 (33)	66 (29)	65 (28)	73 (32)	64 (28)	0.07
Medium	68 (30)	64 (28)	89 (39)	60 (27)	63 (28)	
Fast	84 (37)	97 (43)	74 (33)	94 (41)	100 (44)	
Physical activity (total metabolic equivalents-h/day)						
Energy intake (kcal)	33.7 \pm 2.5	33.7 \pm 2.4	34.3 \pm 4.2	31.9 \pm 2.8	33.8 \pm 3.3	0.73
Nutrient intake	1611 \pm 371	1713 \pm 441	1764 \pm 440	1799 \pm 468	1876 \pm 468	<0.001
Total fat (% energy)	24.9 \pm 4.5	28.0 \pm 4.4	29.8 \pm 4.3	31.1 \pm 4.2	32.8 \pm 4.6	0.03
Saturated fatty acids (% energy)	7.2 \pm 1.8	8.0 \pm 1.7	8.7 \pm 1.8	9.2 \pm 1.8	9.3 \pm 2.0	<0.001
Monounsaturated fatty acids (% energy)	8.7 \pm 1.9	9.9 \pm 1.9	10.5 \pm 1.8	10.8 \pm 2.0	11.0 \pm 2.3	<0.001
Polyunsaturated fatty acids (% energy)	5.8 \pm 1.3	6.4 \pm 1.3	6.6 \pm 1.2	6.6 \pm 1.3	6.6 \pm 1.4	<0.001

[†] Values are the number of subjects (%) or mean \pm standard deviation.

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

Table 3. Metabolic risk factors according to quintile (Q) of total *trans* fatty acid intake ($n = 1136$)^{†‡}

Median (range) value of total <i>trans</i> fatty acid intake (% energy)	Q1 ($n = 227$) 0.56 (0.26-0.65)	Q2 ($n = 227$) 0.72 (0.66-0.78)	Q3 ($n = 228$) 0.88 (0.79-0.94)	Q4 ($n = 227$) 1.03 (0.95-1.13)	Q5 ($n = 227$) 1.32 (1.14-2.25)	<i>p</i> for trend [§]
Body mass index (kg/m ²)						
Model 1 [†]	21.5 ± 0.1	21.4 ± 0.1	21.4 ± 0.1	21.2 ± 0.1	21.2 ± 0.1	0.12
Model 2 ^{††}	21.5 ± 0.1	21.4 ± 0.1	21.4 ± 0.1	21.2 ± 0.1	21.2 ± 0.1	0.17
Model 3 ^{††}	21.4 ± 0.1	21.4 ± 0.1	21.4 ± 0.1	21.2 ± 0.1	21.3 ± 0.1	0.34
Waist circumference (cm)						
Model 1	72.2 ± 0.3	72.3 ± 0.3	73.1 ± 0.3	73.0 ± 0.3	73.9 ± 0.3	<0.001
Model 2	72.3 ± 0.3	72.4 ± 0.3	73.2 ± 0.3	73.0 ± 0.3	73.7 ± 0.3	0.002
Model 3	72.4 ± 0.3	72.3 ± 0.3	73.2 ± 0.3	72.9 ± 0.3	73.6 ± 0.3	0.01
Total cholesterol (mg/dL)						
Model 1	186.8 ± 2.4	186.5 ± 2.2	191.6 ± 2.2	190.5 ± 2.2	190.2 ± 2.3	0.23
Model 2	188.0 ± 2.4	187.4 ± 2.2	192.0 ± 2.2	190.3 ± 2.2	188.0 ± 2.4	0.85
Model 3	187.9 ± 2.4	187.2 ± 2.2	191.8 ± 2.2	190.5 ± 2.2	188.5 ± 2.4	0.74
Low-density lipoprotein cholesterol (mg/dL)						
Model 1	106.1 ± 2.0	104.5 ± 1.8	109.8 ± 1.8	107.4 ± 1.9	107.6 ± 1.9	0.42
Model 2	106.5 ± 2.0	104.8 ± 1.8	110.0 ± 1.8	107.4 ± 1.9	107.8 ± 2.1	0.72
Model 3	106.7 ± 2.0	104.9 ± 1.8	110.0 ± 1.8	107.2 ± 1.9	107.6 ± 2.0	0.87
High-density lipoprotein cholesterol (mg/dL)						
Model 1	69.2 ± 0.9	70.8 ± 0.9	70.6 ± 0.9	71.7 ± 0.9	71.5 ± 0.9	0.12
Model 2	69.8 ± 0.9	71.3 ± 0.9	70.9 ± 0.9	71.5 ± 0.9	70.2 ± 1.0	0.92
Model 3	69.7 ± 0.9	71.2 ± 0.9	70.7 ± 0.9	71.5 ± 0.9	70.7 ± 0.9	0.62
Triacylglycerol (mg/dL)						
Model 1	61.2 ± 2.1	55.7 ± 2.0	62.7 ± 1.9	61.4 ± 2.0	64.8 ± 2.1	0.046
Model 2	61.4 ± 2.2	56.0 ± 2.0	62.7 ± 1.9	61.2 ± 2.0	64.5 ± 2.2	0.10
Model 3	60.9 ± 2.2	55.5 ± 2.0	62.6 ± 1.9	61.4 ± 2.0	65.3 ± 2.1	0.03
Glucose (mg/dL)						
Model 1	84.0 ± 0.5	83.2 ± 0.4	84.2 ± 0.4	84.4 ± 0.4	84.5 ± 0.5	0.14
Model 2	83.9 ± 0.5	83.2 ± 0.4	84.2 ± 0.4	84.4 ± 0.4	84.7 ± 0.5	0.08
Model 3	84.0 ± 0.5	83.3 ± 0.4	84.2 ± 0.4	84.4 ± 0.4	84.5 ± 0.5	0.22
Glycated hemoglobin (%)						
Model 1	4.85 ± 0.02	4.82 ± 0.02	4.88 ± 0.02	4.88 ± 0.02	4.91 ± 0.02	0.003
Model 2	4.85 ± 0.02	4.82 ± 0.02	4.88 ± 0.02	4.88 ± 0.02	4.91 ± 0.02	0.005
Model 3	4.85 ± 0.02	4.82 ± 0.02	4.88 ± 0.02	4.88 ± 0.02	4.90 ± 0.02	0.02

[†] Values are mean ± standard error.

[‡] $n = 1087$ for serum cholesterol (total, low-density lipoprotein, and high-density lipoprotein), triacylglycerol, glucose, and glycated hemoglobin (217 in the first, third, and fifth quintile and 218 in the second and fourth quintile). The median value of *trans* fatty acid intake 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.

^{††} 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), rate of eating (slow, medium, or fast), physical activity (total metabolic equivalents-h/day, continuous), body mass index (kg/m², continuous; except for the analysis of body mass index itself), waist circumference (cm, continuous; except for the analysis of waist circumference itself), and intake of energy (kcal/day, continuous), total fat (% energy, continuous), and saturated fatty acids (% energy, continuous).

^{‡‡} Adjusted for the variables used in model 1, and intake of monounsaturated fatty acids (% energy, continuous) instead of saturated fatty acids.

^{†††} Adjusted for variables used in model 1, and intake of polyunsaturated fatty acids (% energy, continuous) instead of saturated fatty acids.

Table 4. Metabolic risk factors according to quintile (Q) of hydrogenated *trans* fatty acid intake ($n = 1136$)^{†‡}

	Q1 ($n = 227$)	Q2 ($n = 227$)	Q3 ($n = 228$)	Q4 ($n = 227$)	Q5 ($n = 227$)	<i>p</i> for trend [§]
Median (range) value of hydrogenated <i>trans</i> fatty acid intake (% energy)	0.39 (0.09-0.46)	0.53 (0.46-0.59)	0.66 (0.60-0.74)	0.83 (0.74-0.93)	1.11 (0.93-2.18)	
Body mass index (kg/m ²)						
Model 1 [†]	21.4 ± 0.1	21.4 ± 0.1	21.5 ± 0.1	21.3 ± 0.1	21.2 ± 0.1	0.32
Model 2 ^{††}	21.4 ± 0.1	21.3 ± 0.1	21.5 ± 0.1	21.3 ± 0.1	21.3 ± 0.1	0.48
Model 3 ^{†††}	21.3 ± 0.1	21.3 ± 0.1	21.5 ± 0.1	21.3 ± 0.1	21.3 ± 0.1	0.64
Waist circumference (cm)						
Model 1	72.5 ± 0.3	72.6 ± 0.3	72.4 ± 0.3	73.2 ± 0.3	73.8 ± 0.3	0.001
Model 2	72.6 ± 0.3	72.7 ± 0.3	72.5 ± 0.3	73.2 ± 0.3	73.6 ± 0.3	0.02
Model 3	72.7 ± 0.3	72.8 ± 0.3	72.5 ± 0.3	73.1 ± 0.3	73.5 ± 0.3	0.03
Total cholesterol (mg/dL)						
Model 1	185.4 ± 2.3	187.6 ± 2.2	191.1 ± 2.2	191.9 ± 2.2	189.6 ± 2.3	0.23
Model 2	186.4 ± 2.3	188.7 ± 2.2	191.5 ± 2.2	191.8 ± 2.1	187.3 ± 2.3	0.85
Model 3	186.3 ± 2.3	188.3 ± 2.2	191.3 ± 2.1	191.7 ± 2.2	188.0 ± 2.3	0.74
Low-density lipoprotein cholesterol (mg/dL)						
Model 1	104.4 ± 1.9	106.2 ± 1.8	108.8 ± 1.8	109.0 ± 1.8	107.0 ± 1.9	0.34
Model 2	105.0 ± 1.9	106.6 ± 1.9	108.9 ± 1.8	108.9 ± 1.9	106.0 ± 2.0	0.70
Model 3	105.0 ± 1.9	106.7 ± 1.9	108.9 ± 1.8	108.8 ± 1.8	106.0 ± 1.9	0.74
High-density lipoprotein cholesterol (mg/dL)						
Model 1	69.2 ± 0.9	70.4 ± 0.9	71.0 ± 0.9	71.4 ± 0.9	71.8 ± 0.9	0.06
Model 2	69.8 ± 0.9	70.9 ± 0.9	71.2 ± 0.8	71.3 ± 0.8	70.6 ± 0.9	0.62
Model 3	69.7 ± 0.9	70.7 ± 0.9	71.1 ± 0.8	71.3 ± 0.8	71.0 ± 0.9	0.35
Triacylglycerol (mg/dL)						
Model 1	61.3 ± 2.1	58.1 ± 2.0	60.7 ± 1.9	62.5 ± 1.9	63.2 ± 2.0	0.20
Model 2	61.2 ± 2.1	58.3 ± 2.0	60.8 ± 1.9	62.5 ± 1.9	63.0 ± 2.1	0.26
Model 3	61.0 ± 2.1	58.0 ± 2.0	60.6 ± 1.9	62.6 ± 2.0	63.6 ± 2.1	0.14
Glucose (mg/dL)						
Model 1	83.9 ± 0.5	83.8 ± 0.4	83.9 ± 0.4	84.2 ± 0.4	84.6 ± 0.5	0.22
Model 2	83.8 ± 0.5	83.8 ± 0.4	83.9 ± 0.4	84.2 ± 0.4	84.7 ± 0.5	0.14
Model 3	83.9 ± 0.5	83.9 ± 0.4	83.9 ± 0.4	84.2 ± 0.4	84.5 ± 0.5	0.26
Glycated hemoglobin (%)						
Model 1	4.84 ± 0.02	4.85 ± 0.02	4.85 ± 0.02	4.88 ± 0.02	4.91 ± 0.02	0.007
Model 2	4.85 ± 0.02	4.85 ± 0.02	4.86 ± 0.02	4.88 ± 0.02	4.91 ± 0.02	0.01
Model 3	4.85 ± 0.02	4.86 ± 0.02	4.86 ± 0.02	4.88 ± 0.02	4.90 ± 0.02	0.03

[†] Values are mean ± standard error.

^{††} $n = 1087$ for serum cholesterol (total, low-density lipoprotein, and high-density lipoprotein), triacylglycerol, glucose, and glycated hemoglobin (217 in the first, third, and fifth quintile and 218 in the second and fourth quintile). The median value of hydrogenated *trans* fatty acid intake in each quintile was 0.39, 0.54, 0.67, 0.83, and 1.12, respectively.

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

[§] 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 < 1 million, or town and village), survey year (2006 or 2007), current smoking (yes or no), current alcohol drinking (yes or no), rate of eating (slow, medium, or fast), physical activity (total metabolic equivalents-h/day, continuous), body mass index (kg/m², continuous), except for the analysis of body mass index itself, waist circumference (cm, continuous), except for the analysis of waist circumference itself, and intake of energy (kcal/day, continuous), total fat (% energy, continuous), and saturated fatty acids (% energy, continuous).

^{†††} Adjusted for the variables used in model 1, and intake of monounsaturated fatty acids (% energy, continuous) instead of saturated fatty acids.

^{‡‡} Adjusted for variables used in model 1, and intake of polyunsaturated fatty acids (% energy, continuous) instead of saturated fatty acids.

Table 5. Metabolic risk factors according to quintile (Q) of natural *trans* fatty acid intake ($n = 1136$)^{†‡}

	Q1 ($n = 227$)	Q2 ($n = 227$)	Q3 ($n = 228$)	Q4 ($n = 227$)	Q5 ($n = 227$)	<i>p</i> for trend [§]
Median (range) value of natural <i>trans</i> fatty acid intake (% energy)	0.11 (0.01-0.12)	0.15 (0.13-0.16)	0.19 (0.17-0.20)	0.23 (0.21-0.25)	0.32 (0.26-0.64)	
Body mass index (kg/m ²)						
Model 1 [†]	21.3 ± 0.1	21.4 ± 0.1	21.4 ± 0.1	21.4 ± 0.1	21.2 ± 0.1	0.57
Model 2 ^{††}	21.3 ± 0.1	21.4 ± 0.1	21.4 ± 0.1	21.4 ± 0.1	21.2 ± 0.1	0.52
Model 3 ^{†††}	21.2 ± 0.1	21.4 ± 0.1	21.4 ± 0.1	21.4 ± 0.1	21.3 ± 0.1	0.97
Waist circumference (cm)						
Model 1	73.3 ± 0.3	72.9 ± 0.3	72.7 ± 0.3	72.6 ± 0.3	72.9 ± 0.3	0.43
Model 2	73.2 ± 0.3	72.9 ± 0.3	72.7 ± 0.3	72.6 ± 0.3	73.1 ± 0.3	0.84
Model 3	73.3 ± 0.3	73.0 ± 0.3	72.8 ± 0.3	72.6 ± 0.3	72.8 ± 0.3	0.22
Total cholesterol (mg/dL)						
Model 1	187.8 ± 2.4	191.7 ± 2.3	190.3 ± 2.3	190.0 ± 2.3	185.9 ± 2.5	0.40
Model 2	186.9 ± 2.3	191.4 ± 2.2	190.1 ± 2.1	190.3 ± 2.2	186.9 ± 2.3	0.64
Model 3	187.7 ± 2.3	191.8 ± 2.2	190.4 ± 2.1	190.0 ± 2.2	185.8 ± 2.4	0.34
Low-density lipoprotein cholesterol (mg/dL)						
Model 1	105.7 ± 2.0	109.7 ± 1.9	108.8 ± 1.8	109.0 ± 1.8	107.0 ± 2.2	0.41
Model 2	104.8 ± 1.9	109.2 ± 1.8	107.3 ± 1.8	108.6 ± 1.8	105.6 ± 2.0	0.85
Model 3	105.4 ± 2.0	109.6 ± 1.9	107.5 ± 1.8	108.4 ± 1.8	104.6 ± 2.0	0.50
High-density lipoprotein cholesterol (mg/dL)						
Model 1	71.2 ± 0.9	71.3 ± 0.9	71.1 ± 0.8	70.4 ± 0.9	70.0 ± 1.0	0.25
Model 2	70.9 ± 0.9	71.2 ± 0.9	71.0 ± 0.8	70.5 ± 0.9	70.1 ± 0.9	0.44
Model 3	71.1 ± 0.9	71.3 ± 0.9	71.2 ± 0.8	70.4 ± 0.9	69.7 ± 0.9	0.21
Triacylglycerol (mg/dL)						
Model 1	57.1 ± 2.1	61.0 ± 2.0	62.0 ± 1.9	61.9 ± 2.0	63.8 ± 2.3	0.08
Model 2	58.5 ± 2.1	62.1 ± 2.0	62.3 ± 1.9	61.7 ± 2.0	61.2 ± 2.1	0.58
Model 3	58.2 ± 2.1	61.8 ± 2.0	62.2 ± 1.9	61.7 ± 2.0	61.9 ± 2.1	0.39
Glucose (mg/dL)						
Model 1	83.8 ± 0.5	84.3 ± 0.4	83.7 ± 0.4	84.2 ± 0.4	84.3 ± 0.5	0.64
Model 2	83.9 ± 0.5	84.4 ± 0.4	83.8 ± 0.4	84.1 ± 0.4	84.2 ± 0.5	0.85
Model 3	84.0 ± 0.5	84.5 ± 0.4	83.8 ± 0.4	84.1 ± 0.4	84.0 ± 0.5	0.77
Glycated hemoglobin (%)						
Model 1	4.85 ± 0.02	4.89 ± 0.02	4.87 ± 0.02	4.85 ± 0.02	4.88 ± 0.02	0.88
Model 2	4.85 ± 0.02	4.89 ± 0.02	4.87 ± 0.02	4.85 ± 0.02	4.88 ± 0.02	0.87
Model 3	4.86 ± 0.02	4.89 ± 0.02	4.88 ± 0.02	4.85 ± 0.02	4.86 ± 0.02	0.57

[†] Values are mean ± standard error.

^{††} $n = 1087$ for serum cholesterol (total, low-density lipoprotein, and high-density lipoprotein), triacylglycerol, glucose, and glycated hemoglobin (217 in the first, third, and fifth quintile and 218 in the second and fourth quintile). The median value of natural *trans* fatty acid intake in each quintile was the same.

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

^{†††} 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), rate of eating (slow, medium, or fast), physical activity (total metabolic equivalent-h/day, continuous), body mass index (kg/m², continuous; except for the analysis of body mass index itself), waist circumference (cm, continuous; except for the analysis of waist circumference itself), and intake of energy (kcal/day, continuous), total fat (% energy, continuous), and intake of monounsaturated fatty acids (% energy, continuous).

^{††††} Adjusted for the variables used in model 1, and intake of monounsaturated fatty acids (% energy, continuous) instead of saturated fatty acids.

^{†††††} Adjusted for variables used in model 1, and intake of polyunsaturated fatty acids (% energy, continuous) instead of saturated fatty acids.

DISCUSSION

In this group of free-living young Japanese women, total *trans* fatty acid intake was positively associated with waist circumference, triacylglycerol, and HbA1c, but not with BMI, serum cholesterol, or glucose. Hydrogenated *trans* fatty acid intake showed the significant and positive associations with waist circumference and HbA1c, but natural *trans* fatty acid intake did not show any significant association with any of the metabolic risk factors. To our knowledge, this is the first study to examine associations of total, hydrogenated, and natural *trans* fatty acid intake with metabolic risk factors in a free-living young Asian population.

Mean total *trans* fatty acid intake (0.90% of total energy intake) in our Japanese subjects was comparable to that found in one western study (women: 0.95% of total energy intake and men, 0.87%),⁷ but less than those in other western studies (e.g., 1.3% energy-4.3% energy).^{6,8-13,47-49} This variation in intake among studies is likely due to different dietary habits among the populations; the Japanese, for example, have a high intake of rice and fish and lower intake of meat and confectionaries, and consequently a lower fat intake.¹⁵

Regarding the association of total *trans* fatty acid intake and blood lipid as well as glucose metabolic profiles, we found significant positive associations between total *trans* fatty acid intake and triacylglycerol as well as HbA_{1c}, but not with serum cholesterol or glucose. Results of previous studies have been inconsistent. In accordance with our study, LDL and HDL cholesterol was not associated with total *trans* fatty acid intake among 626 adults aged 50-65 years from eight European countries whose intake was similar to that of our present subjects (0.95% versus 0.90% energy), however, the study found an inverse association for total cholesterol.⁷ Consistent with our study, total *trans* fatty acid intake (mean: 1.6% energy) was not associated with total cholesterol among 748 US men aged 43-85 years, but LDL cholesterol, ratio of total cholesterol:HDL cholesterol, and ratio of LDL cholesterol:HDL cholesterol showed positive associations, while HDL cholesterol showed a negative association.⁹ Consistent with our study, HDL cholesterol was not associated with total *trans* fatty acid intake (mean: 6.4 g/day) among 10359 Scottish women aged 40-59 years, but total cholesterol showed a positive association.¹⁰ In addition to this, a positive correlation of *trans* fatty acid intake (mean: 1.3% energy; 1.6% energy; and 1.9% energy respectively) with triacylglycerol was found among 130 men, consisting of lean, lean dyslipidemic, and obese dyslipidemic subjects aged >30 years (mean: 37 years; 41 years; and 40.8 years, respectively) in India, which agrees with our results, but the study also found positive correlations for total cholesterol, LDL cholesterol, and glucose and a negative correlation with HDL cholesterol.¹² As with our study, no correlation of *trans* fatty acid intake (mean 2.7% of energy in women, 3.5% in men) with glucose was observed among 38 American adults with a range of glucose tolerance aged >18 years (mean: 34 years); the study also found no correlation for insulin, ratio of insulin:glucose area under the curve during the oral glucose tolerance test, or in homeostasis model assessment insulin resistance.⁸ These discrepancies among observational

results are likely explained by the different dietary habits among populations, different intake of *trans* fatty acids, and different biomarkers.

With regard to the association of total *trans* fatty acid intake and measures of obesity, we found a significant positive association between total *trans* fatty acid intake and waist circumference, but not with BMI. Consistent with our study, positive associations of *trans* fatty acid intake with waist circumference but not with BMI was found among 130 men in India.¹² The study also found positive associations with waist-hip ratio and the conicity index, a measure of abdominal obesity. Further, a 9-year prospective study among 16587 American men aged 40-75 years found a positive association between *trans* fatty acid intake (mean: 1.3% energy in subjects aged 50-75 years; and 1.4% in those aged 40-49 years) and waist gain.¹¹ The study found that replacement of *trans* fatty acids as 2% of energy intake for polyunsaturated fats was associated with a 0.77 cm waist gain over 9 years. We found that the difference in terms of waist size of the subjects in the extreme quintile of total *trans* fatty acid intake (median 0.56% energy in quintile 1 versus 1.32% energy in quintile 5) was 1.2-1.7 cm. Although not directly comparable, the two results may suggest a considerable effect of *trans* fatty acid intake on waist circumference. In contrast, an 8-year prospective study on 41518 American women aged 41-68 years found a positive association between *trans* fatty acid intake (mean: 1.7% energy) and weight gain.¹³ Given the limitation in terms of evidence, more studies are warranted in order to make conclusion on the associations.

Possible mechanisms of the adverse effect of *trans* fatty acids on cholesterol levels include inhibition of lecithin:cholesterol acyltransferase activity, decreased rates of LDL apoB-100 catabolism, increased rates of apoA1 catabolism, reduced LDL cholesterol particle size, and increased plasma activity of cholesteryl ester transfer protein, the main enzyme in the transfer of cholesterol esters from HDL to LDL and very low-density lipoprotein cholesterol.^{50,51} In terms of insulin sensitivity, *trans* fatty acids may inhibit delta-5 desaturase activity,^{52,53} alter the skeletal muscle cell membrane structure,⁵⁴ decrease adipocyte plasma membrane fluidity,⁵⁵ and alter concentrations of interleukin 6, tumor necrosis factor, and prostaglandins.⁵⁶ The resulting decrease in insulin sensitivity would then influence waist circumference by increasing lipoprotein lipase activity in abdominal adipose tissue due to higher cellularity and blood flow.^{11,57} Clarification of the relationship between *trans* fatty acid intake and metabolic risk factors requires additional input from observational studies among free-living populations, particularly Asian populations.

In the analyses examining an association of hydrogenated or natural *trans* fatty acid intake with metabolic risk factors, we found significant positive associations only between hydrogenated *trans* fatty acid intake and waist circumference as well as HbA_{1c} (*p* for trend ≤ 0.03 ; models 1-3). Natural *trans* fatty acid was not associated with any of the metabolic risk factors. Natural *trans* fatty acids largely contain vaccenic acid (C18:1 ω -11, *trans*) while hydrogenated *trans* fatty acids mainly contain elaidic acid (C18:1 ω -9, *trans*), which may interfere with

metabolism of essential fatty acids.⁵⁸ Results among 10359 Scottish women aged 40-59 years, however, showed no association of hydrogenated *trans* fatty acids intake with either total or HDL cholesterol, while a positive association between natural *trans* fatty acid intake and total cholesterol was found.¹⁰ The null association for natural *trans* fatty acids observed in our study may be due to the narrow ranges of natural *trans* fatty acid intake among study subjects. More studies examining associations of *trans* fatty acid intake based on dietary sources with metabolic risk factors among various age groups are warranted.

Several limitations of the present study should be acknowledged. First, the use of a self-administered, semi-quantitative dietary assessment questionnaire (i.e., DHQ)¹⁹⁻²² is subject to measurement error in dietary intake, and actual dietary habits were not obtained. Additionally, the DHQ covers a limited number of foods, and analytic *trans* fatty acid values were not available for all foods covered. Moreover, variation in values among food products within the same food group was also recognized.²⁸⁻⁴³ Particularly, several western countries have taken efforts to reduce *trans* fatty acid contents in foods in recent decades; thus, such efforts may have been taken in Japan. However, there has been no regulation (e.g., food labeling or recommended upper limit intake level) in Japan. Data on *trans* fatty acid content of margarine sold in Japan published from 1998 to 2006 showed no clear trend of reduction; mean *trans* fatty acid value was 11.1% of fat content (range: 1.5-21.5%) in 1998 ($n = 15$)³⁵; 11.7% (2.3-19.5%) in 2002 ($n = 13$)³⁶; 9.9% (1.1-17%) in 2006 ($n = 27$)²⁸; and 10.1% (0.43-16.4%) in 2006 ($n = 20$).⁴² Although we cannot verify that such circumstances has occurred in other foods as well, margarine is one of the representative foods containing high amount of *trans* fatty acids and used for making bakery goods and confectioneries. Thus, we included data on *trans* fatty acid contents in foods published in the 1990's. Nevertheless, we acknowledge that the database is likely not complete. Although correlations between the DHQ and dietary records were reasonable, suggesting its suitability for *trans* fatty acid research, validation of the DHQ using biomarkers of *trans* fatty acid intake (e.g., adipose tissues and erythrocytes) is likely preferable given that *trans* fatty acids are not endogenously produced. Although not identical, Pearson correlation coefficients between the DHQ and serum concentrations were 0.66 for eicosapentaenoic acid and docosahexaenoic acid and 0.36 for alpha-linolenic acid in 44 Japanese women.²¹

Second, the cross-sectional nature of the study does not allow the establishment of cause-effect relationships due to uncertain temporality of the associations.

Third, the sample population consisted of selected female students who enrolled in dietetic courses, and may therefore have had relatively healthy lifestyles. Thus, the clinical relevance of our findings remains to be elucidated. Also, since we recruited subjects who responded positively to enrollment after a brief explanation of the study outline, the exact response rate was unknown and selection bias may be present, limiting the generalizability of the results. Nevertheless, our subjects were on average similar to a representative sample of Japanese women

aged 20-29 years, at least with regard to daily intake of energy (mean: 1673 kcal/day) and total fat (mean: 29.0% energy) and the measurement of several metabolic risk factors, including BMI (mean: 20.3 kg/m²), total cholesterol (mean: 179 mg/dL), HDL cholesterol (mean: 69 mg/dL), and HbA1c (mean: 4.8%) (data not available for *trans* fatty acid intake, waist circumference, LDL cholesterol, triacylglycerol, and glucose).¹⁵

Fourth, due to correlations among subtypes of fatty acids, we were unable to evaluate the effect of replacing energy from *trans* fatty acids with the same amount of energy from other fatty acid subtypes (Pearson's correlation coefficients: 0.60 for saturated fatty acids and monounsaturated fatty acids, 0.83 for monounsaturated fatty acids and polyunsaturated fatty acids, and 0.23 for saturated fatty acids and polyunsaturated fatty acids).

Finally, notwithstanding our comprehensive control of major confounding factors, the possibility of residual confounding cannot be excluded.

In conclusion, we found that *Trans* fatty acid intake was positively associated with several metabolic risk factors among free-living young Japanese women with relatively low intake. The associations seemed to be largely explained by hydrogenated *trans* fatty acid intake, since they accounted for 77% of total intake. Since the cross-sectional nature of the study precludes causal inferences, further observational studies to clarify the relationship between *trans* fatty acid intake and metabolic risk factors are required.

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APPENDIX

Additional study group members are Mitsuyo Yamasaki, Yuko Hisatomi, Junko Soezima, and Kazumi Takedomi (Nishikyushu University); Toshiyuki Kohri and Naoko Kaba (Kinki University); Etsuko Uneoka (Otemae College of Nutrition); Hitomi Hayabuchi and Yoko Umeki (Fukuoka Women's University); Keiko Baba and Maiko Suzuki (Mie Chukyo University Junior College); Reiko Watanabe and Kanako Muramatsu (Niigata Women's College); Kazuko Ohki, Seigo Shiga, Hidemichi Ebisawa, and Masako Fuwa (Showa Women's University); Tomoko Watanabe, Ayuho Suzuki, and Fumiyo Kudo (Chiba College of Health Science); Katsumi Shibata, Tsutomu Fukuwatari, and Junko Hirose (The University of Shiga Prefecture); Toru Takahashi and Masako Kato (Mimasaka University); Toshinao Goda and Yoko Ichikawa (University of Shizuoka); Junko Suzuki, Yoko Niida, Satomi Morohashi, Chiaki Shimizu, and Naomi Takeuchi (Hokkaido Bunkyo University); Jun Oka and Tomoko Ide (Tokyo Kasei University); and Yoshiko Sugiyama and Mika Furuki (Minamikyushu University).

AUTHOR DISCLOSURES

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