Methods

Subjects

The present study was based on a survey conducted in three areas of Japan (i.e. Osaka (urban), Nagano (rural inland) and Tottori (rural coastal)). Detailed descriptions of the survey have been published elsewhere.[18] Briefly, apparently healthy women aged 30–69 years who were willing to participate with their husbands were recruited in each area, such that each 10-year age class (30–39, 40–49, 50–59 and 60–69 years) contained eight women equally (without consideration of the age of the men), giving a total of 96 women and 96 men invitees. Group orientations for the subjects were held prior to the study, at which the study purpose and protocol were explained. Written informed consent was obtained from each subject. A total of 92 women aged 31–69 years and 92 men aged 32–76 years completed the study protocol and were included in the present analysis. Basic characteristics of the 92 women and 92 men have been described elsewhere.[18]

Dietary assessment

Between November 2002 and September 2003, the subjects completed the DHQ (assessing diet during the preceding month) and the 4-nonconsecutive-day weighed DRs (one weekend day and three weekdays) four times (once per season) at intervals of approximately three months (DHQ1 in November 2002 (autumn), DHQ2 in February 2003 (winter), DHQ3 in May 2003 (spring) and DHQ4 in August and September 2003 (summer) and DR1 in November and December 2002 (autumn), DR2 in February 2003 (winter), DR3 in May 2003 (spring) and DR4 in August and September 2003 (summer)). In each season, the DHQ was completed before the start of the dietary recording period. An additional DHQ (DHQ5) was also completed about one year after completing DHQ1 (in November 2003 (autumn)).

Detailed descriptions of the DRs have been published elsewhere.[18] Briefly, the subjects were asked to record and weigh all foods and drinks consumed on each recording day, and then to fax the completed records to the local staff (registered dietitians). The submitted forms were reviewed by the staff and, if necessary, the subjects were asked to add or modify the records by telephone or fax. The coding of records and conversion of other measurements of quantities into grams were performed by trained registered dietitians in the survey center in accordance with uniform procedures. A total of 1299 food and beverage items appeared in the DR. Estimates of daily energy intake were calculated based on the Standard Tables of Food Composition in Japan.[19]

Detailed descriptions of the DHQ have also been published elsewhere.[18,20–22] Briefly, the DHQ is a 16-page structured questionnaire that assesses dietary habits during the preceding month (i.e. the consumption frequency and portion size of selected foods commonly consumed in Japan as well as general dietary behaviour and usual cooking methods).[20] Responses to the DHQ were checked at least twice for completeness by the local staff, and when necessary reviewed with the subject to ensure the clarity of answers. Estimates of daily intake for foods (150 items in total) and energy were calculated using an ad hoc computer algorithm for the DHQ[18,20] based on the Standard Tables of Food Composition in Japan.[19]

Calculation of monetary diet cost

For both the DR and DHQ, monetary diet cost (Japanese yen/day) was calculated by multiplying the amount of each food reported (g/day) by the estimated price of the food (Japanese yen/g) and then summing the products (1 Japanese yen = 0.0047 pound sterling = 0.0059 euros = 0.0094 U.S. dollars in July 2008). The procedure for estimating costs was based on the assumption that all foods were purchased and then prepared and consumed at home.[12,14] Calculations included correction for preparation and waste (e.g. trimming and peeling of vegetables and fruits, removal of bones and skin from fish).[6,14] Costs of combined foods such as pizza were calculated using the prices of frozen equivalents.[14,15] Water was excluded from calculation (two items in the DR and three items in the DHQ).[11,14] The price of foods was obtained from two sources. The first was the National Retail Price Survey 2004.[23] This survey was conducted in 167 villages, towns and cities, and average prices were calculated as mean values of all survey areas, weighted for population size. The second source was information on price from the websites of nationally distributed supermarket (Seiyu) and fast-food restaurant (McDonalds and Mister Donut) chains. When more than one price was available from the websites, the mean value was used.

To determine the price of individual food items, each food in the DR and DHQ was directly matched to foods appearing in the National Retail Price Survey. This procedure was used to determine the price of 656 of the 1297 items used in the DR (51%) and 120 of the 147 items used in the DHQ (82%). A total of 605 of the remaining 641 items in the DR for which a price value was not available in the National Retail Price Survey but which had a comparable food in terms of price (according to information on the websites) appearing in the National Retail Price Survey (47%) were assigned a value according to the comparable food. This procedure was also used to determine the price of 13 of the remaining 27 items in the DHQ (9%). For the remaining 36 items in the DR (3%) and 14 in the DHQ (10%) which had no price value and no comparable food in the National Retail Price Survey, prices were taken from the websites.

As the treatment of alcoholic beverages and noncaloric beverages in the calculation of monetary diet cost varies among studies,[3–17] we used the following four calculation strategies: 1) all foods and beverages included; 2) alcoholic beverages excluded; 3) noncaloric beverages excluded; and 4) both alcoholic and noncaloric beverages excluded. Mean contributions to energy intake of the foods for which a price value was directly determined from the National Retail Price Survey, the foods which were assigned the price of a comparable food in the National Retail Price Survey, and the foods for which a price value was taken from the websites were 87%–91%, 5%–11%, and 2%–5%, respectively, depending on sex, dietary assessment method, and calculation strategy. The corresponding values for monetary diet cost were 81%–95%, 2%–17%, and 2%–6%, respectively.

While the misreporting of dietary intake, particularly by overweight subjects, is a serious problem associated with self-report dietary assessment methods,[24] body mass index-dependent misreporting seems to be canceled by energy-adjustment, at least for potassium, sodium, and protein estimated from the DHQ.[25] Thus, energy-adjusted values of monetary diet cost (by the residual and density models)[26] were used in the present study. Because the results based on the residual model were quite similar to those based on the density model, we only present the results based on the energy-adjusted value of monetary diet cost by the density model (i.e. monetary cost of dietary energy (Japanese yen/4184 kJ)). The monetary cost of dietary energy of each food item in the DHQ (as well as the categorization of food groups) has been published elsewhere,[14] except for the following 13 food items: three kinds of ice cream (regular 425, premium 959, and unspecified varieties 658 Japanese yen/4184 kJ), six alcoholic beverages (beer 1465, sake 746, shochu 497, shochu mixed with water or a carbonated beverages 533, whiskey 588, and wine 1235 Japanese yen/4184 kJ), and four noncaloric beverages (green and oolong tea 11, black tea 17, coffee 18, and sugar-free soft drinks 24 Japanese yen/100 g of edible weight).

Statistical analysis

All statistical analyses were performed for women and men separately using SAS statistical software version 8.2 (SAS Institute Inc., Cary, NC, U.S.A). Distributions of monetary cost of dietary energy were evaluated for deviations from normality; because the variable was not strongly skewed, untransformed values were used. Mean and SD values for monetary cost of dietary energy were calculated for both DRs and DHQs. To assess seasonal variation, intraclass correlations were calculated using DRs (DR1, DR2, DR3 and DR4) and DHQs (DHQ1, DHQ2, DHQ3 and DHQ4) conducted in each season over a 1-year period. Intraclass correlations were also calculated between DHQs completed in the same season about one year apart (DHQ1 and DHQ5) to assess reproducibility of the DHQ.

To assess the comparability of the DR and DHQ, Pearson correlations between the mean of DRs1-4 and mean of DHQs1-4 were calculated. Pearson correlations were also calculated between the mean of DRs1-4 and DHQ1 to examine whether the DHQ (assessing dietary habits during the preceding month) is able to capture monetary cost of dietary energy over a longer period (i.e. one year). We used DHQ1 for this purpose because the answers provided in the other DHQs (administered after gaining experience of the DRs), but not DHQ1 (administered before this experience), may have been influenced by the attention to diet required to complete the DRs. Since random within-individual error in the measurement of any of the variables being compared tends to reduce correlation coefficients toward zero,[27] correlations with the corrections for the attenuating effects of such

measurement error in the 4 × 4-day DRs were also computed, as described elsewhere.[18] Additionally, we calculated the percentage of subjects who were classified in the same, adjacent, or opposite quintile of monetary cost of dietary energy in the two different assessment methods. Further, the agreement between the two methods was assessed by the method proposed by Bland and Altman,[28] using a plot of the difference between the two methods against the average of the two methods.

Results

As shown in Table 1 (for DRs) and Table 2 (for DHQs), monetary cost of dietary energy was calculated from both dietary assessment methods conducted in each season over one year (DR1, DR2, DR3 and DR4 and DHQ1, DHQ2, DHQ3 and DHQ4) for assessing seasonal variations. Mean differences were within 6% for DRs and 9% for DHQs, and intraclass correlations ranged from 0.52 to 0.63 for DRs and 0.54 to 0.66 for DHQs. To assess the reproducibility of DHQ, the intraclass correlations between DHQs completed one year apart (DHQ1 and DHQ5) was calculated (Table 2). The intraclass correlations ranged from 0.50 to 0.64, with mean differences of less than 1%.

Comparability of the DR and DHQ for estimating monetary cost of dietary energy was assessed by using the value derived from DRs1- 4 and that derived from DHQs1- 4 (Table 3). Mean differences between DRs1-4 and DHQs1- 4 were within 8%. The Pearson correlations between DRs1- 4 and DHQs1- 4 ranged from 0.60 to 0.71. The percentage of subjects categorized into the same or adjacent quintiles was more than 71%, while the percentage categorized into the opposite quintile was less than 3%. Comparison of the first DHQ (DHQ1) with DRs1- 4 was also conducted to examine whether the DHQ (assessing dietary habits during the preceding month) is able to capture monetary cost of dietary energy over a longer period (i.e. one year) (Table 3). Mean differences between DRs1- 4 and DHQ1 were within 10% and Pearson correlations ranged from 0.41 to 0.61, while the percentage of subjects categorized to the same or adjacent and opposite quintiles was more than 61% and less than 4%, respectively.

Bland-Altman plots assessing the agreement between DRs1- 4 and DHQs1- 4 for monetary cost of dietary energy (calculated based on all foods and beverages) are shown in Figure 1. The mean difference (95% CI) between the two methods (DRs1- 4 minus DHQs1- 4) was 23.6 (9.2, 38.1)

Table 1. Monetary cost of dietary energy (Japanese yen/4184 kJ) estimated from 4-day weighed dietary records (DRs) conducted in each season over one year (DR1, DR2, DR3 and DR4) and intraclass correlation (*r*) in 92 Japanese women and 92 Japanese men[a].

	DR1[b]		DR2[c]		DR3[d]		DR4[e]		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Intraclass *r*
Women									
Including all foods and beverages	582	97	573	97	583	94	603	111	0.63
Excluding alcoholic beverages	572	92	564	93	574	91	591	107	0.60
Excluding noncaloric beverages	545	95	537	94	543	91	561	109	0.62
Excluding both alcoholic and noncaloric beverages	535	90	527	90	534	88	549	103	0.59
Men									
Including all foods and beverages	575	97	558	98	569	102	594	98	0.59
Excluding alcoholic beverages	549	96	536	92	540	91	558	89	0.52
Excluding noncaloric beverages	544	97	528	96	535	104	559	100	0.61
Excluding both alcoholic and noncaloric beverages	515	96	503	88	504	90	519	88	0.53

[a]1 Japanese yen = 0.0047 pound sterling = 0.0059 euros = 0.0094 U.S. dollars in July 2008.
[b]Conducted in November and December 2002 (autumn).
[c]Conducted in February 2003 (winter).
[d]Conducted in May 2003 (spring).
[e]Conducted in August and September 2003 (summer).

Table 2. Monetary cost of dietary energy (Japanese yen/4184 kJ) estimated from self-administered diet history questionnaires (DHQs) conducted in each season over one year (DHQ1, DHQ2, DHQ3 and DHQ4) and that conducted one year after completion of DHQ1 (DHQ5), and intraclass correlation (*r*) in 92 Japanese women and 92 Japanese men[a].

	DHQ1[b]		DHQ2[c]		DHQ3[d]		DHQ4[e]		DHQ5[f]		Intraclass *r*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	DHQs1-4	DHQ1 and DHQ5
Women												
Including all foods and beverages	562	92	540	84	558	80	586	88	558	86	0.66	0.64
Excluding alcoholic beverages	550	83	531	80	545	80	574	88	548	84	0.63	0.61
Excluding noncaloric beverages	505	87	489	84	503	81	535	88	504	85	0.66	0.64
Excluding both alcoholic and noncaloric beverages	492	80	479	80	489	80	522	84	493	84	0.64	0.62
Men												
Including all foods and beverages	555	99	548	99	544	97	591	92	556	95	0.59	0.51
Excluding alcoholic beverages	512	82	506	90	504	80	546	74	513	80	0.54	0.50
Excluding noncaloric beverages	511	99	507	95	499	100	548	92	515	97	0.64	0.57
Excluding both alcoholic and noncaloric beverages	463	81	459	84	454	80	498	76	468	80	0.61	0.57

[a]1 Japanese yen = 0.0047 pound sterling = 0.0059 euros = 0.0094 U.S. dollars in July 2008. DHQ is designed to assess dietary habits during the preceding month.
[b]Conducted in November 2002 (autumn).
[c]Conducted in February 2003 (winter).
[d]Conducted in May 2003 (spring).
[e]Conducted in August and September 2003 (summer).
[f]Conducted in November 2003 (autumn).

Table 3. Monetary cost of dietary energy (Japanese yen/4184 kJ) estimated from 4-day weighed dietary records (DRs) and self-administered diet history questionnaires (DHQs) conducted in each season over one year (mean of DRs1-4 and mean of DHQs1-4, respectively) and the Pearson correlation (*r*) and percentage of subjects classified in the same, adjacent, and opposite quintiles between the mean of DRs1-4 and that of DHQs1-4 and between mean of DRs1-4 and the first DHQ (DHQ completed before DRs; DHQ1) in 92 Japanese women and 92 Japanese men[a].

	Mean of DRs1-4[b]		Mean of DHQs1-4[c]		Mean of DRs1-4 and mean of DHQs1-4					Mean of DRs1-4 and DHQ1				
					Pearson *r*		Cross-classification (%)			Pearson *r*		Cross-classification (%)		
	Mean	SD	Mean	SD	Crude	Corrected[d]	Same quintile	Adjacent quintile	Opposite quintile	Crude	Corrected[d]	Same quintile	Adjacent quintile	Opposite quintile
Women														
Including all foods and beverages	585	85	561	76	0.63	0.64	38	38	0	0.59	0.60	36	37	1
Excluding alcoholic beverages	575	81	550	71	0.59	0.61	42	34	0	0.55	0.56	41	33	1
Excluding noncaloric beverages	547	83	508	75	0.66	0.67	42	34	0	0.60	0.61	39	32	1
Excluding both alcoholic and noncaloric beverages	536	77	496	71	0.62	0.63	42	29	0	0.55	0.57	37	37	1
Men														
Including all foods and beverages	573	82	561	83	0.68	0.69	42	36	0	0.51	0.52	29	42	1
Excluding alcoholic beverages	545	74	517	68	0.58	0.60	33	41	1	0.40	0.41	27	35	3
Excluding noncaloric beverages	541	83	517	85	0.70	0.71	37	42	0	0.56	0.57	32	43	1
Excluding both alcoholic and noncaloric beverages	509	73	469	70	0.58	0.60	28	49	2	0.45	0.46	27	39	3

[a]1 Japanese yen = 0.0047 pound sterling = 0.0059 euros = 0.0094 U.S. dollars in July 2008. DHQ is designed to assess dietary habits during the preceding month.
[b]Conducted in November and December 2002 (autumn), February 2003 (winter), May 2003 (spring) and August and September 2003 (summer).
[c]Conducted in November 2002 (autumn), February 2003 (winter), May 2003 (spring) and August and September 2003 (summer).
[d]Corrected for seasonal variation in DRs.

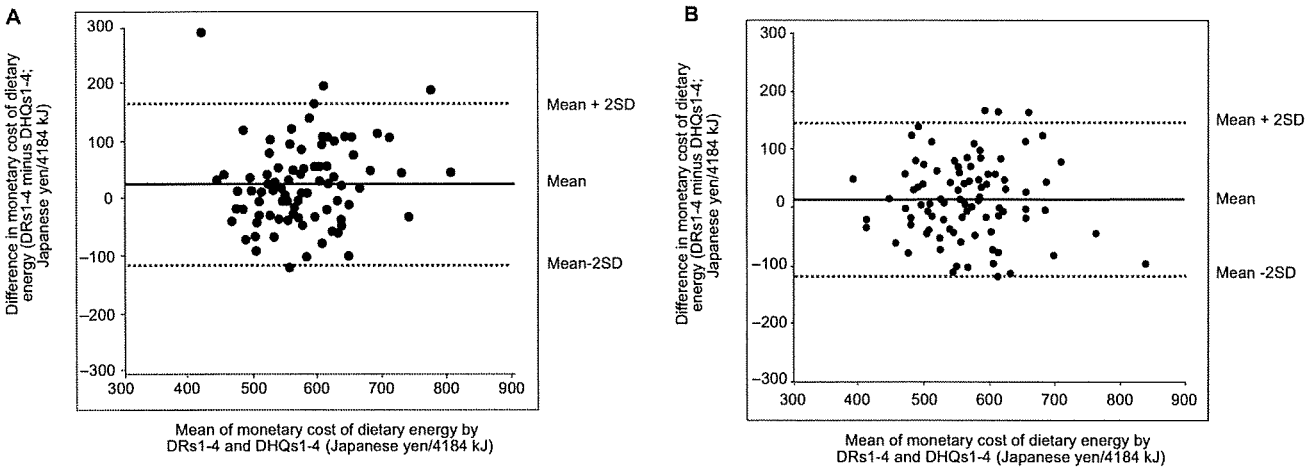

Figure 1. Bland-Altman plots assessing the agreement between 4-day weighed dietary records (DRs) and self-administered diet history questionnaires (DHQs) conducted in each season over one year (mean of DRs1-4 and mean of DHQs1-4, respectively) for monetary cost of dietary energy (calculated based on all foods and beverages) in 92 Japanese women (**a**) and 92 Japanese men (**b**).

Japanese yen/4184 kJ for women and 12.8 (−0.9, 26.6) Japanese yen/4184 kJ for men, indicating relatively good agreement at the group level. The limits of agreement (mean difference ± 2SD of the difference) ranged from −115.6 to 162.9 Japanese yen/4184 kJ for women and −120.0 to 145.7 Japanese yen/4184 kJ for men, indicating somewhat moderate to poor agreement at the individual level. The plots indicated no tendency of consistent bias. Similar plots were observed when different cost calculation strategies or DHQ1 rather than DHQs1-4 were used (data not shown).

Important contributors to total monetary diet cost (based on DRs1-4 and DHQs1-4) were vegetables (12.0%–19.4%), fish and shellfish (17.0%–19.2%), meat (11.5%–12.8%), and noncaloric beverages (6.5%–10.0%), followed by confectioneries (4.3%–9.0%), fruits (5.1%–7.8%) and rice (5.8%–6.8%). In men, alcoholic beverages were also important contributors (12.6%–16.1%).

Discussion

The present study of 92 Japanese women and 92 Japanese men showed reasonable comparability of monetary cost of dietary energy across DR and a DHQ for Japanese adults. Additionally, even a single administration of our DHQ (assessing dietary habits during the preceding month) appeared to relatively reasonably capture monetary cost of dietary energy over a longer period (i.e. one year), seemingly due to a relatively small seasonal variation in monetary cost of dietary energy as well as good reproducibility of DHQ. Because this is the first study to examine the comparability of monetary diet cost across dietary assessment methods, comparison of our results with others cannot be readily made. However, the comparability of DHQ and DR for estimating monetary cost of dietary energy observed here was similar to that for nutritional factors commonly studied in epidemiological studies with the use of dietary assessment questionnaires.[26]

The major contribution to total monetary diet cost in the present study came from perishable fresh foods such as vegetables, fish and shellfish, and meat. Consistent findings have been observed in several previous studies.[14–16] This is reasonable given that transport, storage, and wastage costs are all high for perishable fresh produce. Although the question of whether alcoholic and noncaloric beverages should be included in the calculation of monetary diet cost has not been answered,[3–17] the contribution of these beverages in the present study was not small. The treatment of these beverages in future research should thus be carefully considered, although the comparability of monetary cost of dietary energy here did not materially differ irrespective of the treatment of these beverages.

Several limitations of the present study should be mentioned. First, because of a lack of the true measure of monetary diet cost (i.e. actual food expenditure data), the present study unfortunately provides no information on the validity of monetary diet cost estimated based on food intake data derived from dietary assessment methods. Alternatively, the present study only provides

information on the comparability of a DHQ and DR for estimating monetary diet cost. Thus, future investigation on the validity of monetary diet cost estimated from dietary intake data against true measure of monetary diet cost (e.g. a shopping diary and the collection of grocery till receipts supplemented by the recording of actual food consumption) is needed, although obtaining an accurate measure of food expenditure data at the individual level seems to be somewhat challenging.[12]

Second, both dietary assessment methods used in the present study (i.e. DR and DHQ) are not free from measurement error. However, it should be noted that errors in DR are thought to have lesser correlation with errors in DHQ, because the major sources of error associated with DHQ are limited food items, memory of food consumed, assessment of portion size, and interpretation of questions, while these sources of potential error are minimally shared with the DR method, which is open-ended, involves recording of foods as they are consumed, and involves direct weighing of food portions.[26]

Third, food prices were derived from the National Retail Price Survey and websites of nationally distributed supermarket and fast-food restaurant chains. Because this procedure provides a single cost value for a given food, without consideration of local, regional, or between-subject variations, it provides only an approximation of actual diet costs. Errors in the price values for foods will be shared by the DR and DHQ and may increase the observed correlations. Although this characteristic is common to standard nutrient databases, the actual diet cost may depend on where people live and shop, the number of people in the household (e.g. higher prices for the same food item for small households due to smaller packet size), or the extent to which people eat out at restaurants and takeaways.[16] However, it should be noted that a similar methodology has been used in all previous studies[4–17] with only one exception,[3] as mentioned above.

Finally, the generalizability of these results may be limited, because the study evaluated one particular DHQ designed for use in Japan. Further, the sample size of this study was relatively small, and the subjects were not a representative sample of general Japanese but rather volunteers. Additional studies for other dietary assessment techniques in other populations would add valuable information on this topic.

To conclude, the present data indicate the reasonable comparability of monetary cost of dietary energy across DR and a DHQ for Japanese adults as well as the usefulness of a single administration of the DHQ for estimating monetary cost of dietary energy. The present findings may lend support to the practice of using dietary assessment questionnaires to estimate monetary diet cost.

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Disclosure

The authors report no conflicts of interest.

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Research articles

Lower estimates of δ-5 desaturase and elongase activity are related to adverse profiles for several metabolic risk factors in young Japanese women

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Abstract

Little is known about the relation between the activities of certain enzymes involved in endogenous fatty acid synthesis and metabolic risk factors, particularly in young adults and non-Western populations. In this cross-sectional study, we examined the associations between estimated desaturase and elongase activities and metabolic risk factors in young Japanese women. The subjects were 640 female Japanese dietetic students aged 18 to 22 years. Body height and weight, from which body mass index (BMI) was derived, waist circumference, and blood pressure were measured. Fasting blood samples were collected for biochemical and fatty acid measurements. Desaturase and elongase enzyme activities were estimated as the ratio of product to precursor of individual fatty acids in serum lipids. δ-9 desaturase activity was positively associated with BMI, diastolic blood pressure, total and low-density lipoprotein cholesterol, and triacylglycerol and was negatively associated with high-density lipoprotein cholesterol ($P \leq .019$). δ-6 desaturase activity showed positive associations with BMI, systolic blood pressure, triacylglycerol, and the homeostasis model assessment of insulin resistance ($P \leq .045$). δ-5 desaturase activity showed independent negative associations with BMI, systolic blood pressure, triacylglycerol, insulin, and the homeostasis model assessment of insulin resistance ($P \leq .007$). Elongase activity was associated negatively with BMI, systolic and diastolic blood pressures, and triacylglycerol and was positively associated with high-density lipoprotein cholesterol ($P \leq .026$). In conclusion, increased estimates of δ-9 and δ-6

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desaturase activity and decreased estimates of δ-5 desaturase and elongase activity were associated with adverse profiles for several metabolic risk factors in young Japanese women.

Keywords: Desaturase; Elongase; Metabolic risk factors; Young Japanese women; Epidemiology

Abbreviations: BMI, body mass index; D5D, δ-5 desaturase; D6D, δ-6 desaturase; D9D, δ-9 desaturase; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.

1. Introduction

A number of enzymes are involved in the endogenous synthesis of fatty acids, including δ-9 desaturase (D9D), δ-6 desaturase (D6D), δ-5 desaturase (D5D), and elongase [1]. δ-9 desaturase, D6D, and D5D introduce a double bond at specific positions (the ninth, sixth, and fifth carbons from the carboxyl terminal, respectively) of long-chain fatty acids [2]; D9D synthesizes monounsaturated fatty acids from saturated fatty acids, whereas D6D and D5D catalyze the synthesis of long-chain n-6 and n-3 polyunsaturated fatty acids [1]. Elongase, which inserts 2 carbon units at the carboxyl terminal of the fatty acid [2], is involved in many aspects of endogenous fatty acid synthesis [1]. The activity of these enzymes can be reasonably estimated from the product precursor ratios of individual fatty acids in human tissues such as serum lipids [3-5].

A body of evidence from a limited number of observational studies of free-living populations suggests that high estimates of D9D and D6D activity and low estimates of D5D and elongase activity are generally associated with unfavorable metabolic risk factor profiles [3-11]. However, association with each of metabolic risk factors was poorly examined. In relatively large studies, association with only one risk factor or combination of metabolic risk factors (ie, metabolic syndrome) was examined [3,5,7,11], whereas other studies examining multiple risk factors had methodological limitations such as a small number of subjects, no consideration of confounding factors, or both [4,6,8-10].

In addition, research among young adult populations is sparse. Identification of patterns of desaturase and elongase activity associated with metabolic risk factors in young adult populations is vitally important; however, not only because an adverse metabolic risk factor profile, characterized by metabolic syndrome, is an independent predictor of cardiovascular diseases [12,13] and type 2 diabetes [12,14], but also because the findings in young people are usually not confounded by metabolic disorders.

Furthermore, research among non-Western populations, including Japanese, is sparse [11]. Dietary and serum fatty acid composition, which not only can alter but also can reflect desaturase and elongase activities [1], differs between Japanese and Western people. This is mainly because of the higher consumption of n-3 long chain polyunsaturated fatty acids derived from marine products such as eicosapentaenoic acid and docosahexaenoic acid by Japanese than most Western people (mean fish intake, 71 and 32 g/d [15]; mean intake of eicosapentaenoic acid plus docosahexaenoic acid, 0.46% and 0.07% of energy intake [16], respectively) [17]. On this basis, Japanese and Western populations may differ in the associations of activities of enzymes involved in endogenous fatty acid synthesis with metabolic risk factors.

The aim of this cross-sectional study of young Japanese women was to investigate the associations of estimated activities of enzymes involved in endogenous fatty acid synthesis, including D9D, D6D, D5D, and elongase, with several metabolic risk factors. We hypothesized that higher estimates of D9D and D6D activity and lower estimates of D5D and elongase activity are associated with adverse profiles for metabolic risk factors.

2. Methods and materials

2.1. Subjects

The present study was based on a multicenter survey conducted from January to March 2007 among female dietetic students from 11 institutions in Japan. All measurements at each institution were conducted according to the survey protocol. Briefly, staff at each institution explained an outline of the survey to potential subjects. Those responding positively were then provided detailed written and oral explanations of the general purpose and procedure of the survey. A total of 702 Japanese women took part. For the present analysis, we selected 640 women who met the following 4 inclusion criteria: age 18 to 22 years (n = 687); no previous diagnosis of diabetes, hypertension, or cardiovascular disease (n = 701); provision of fasting blood samples (n = 663); and no missing information on variables used in the present study (n = 701). Written informed consent was obtained from each subject and also from a parent for subjects younger than 20 years. The protocol of the study was approved by the Ethics Committee of the National Institute of Health and Nutrition, Tokyo, Japan.

2.2. Blood sampling

Peripheral blood samples were obtained from subjects after an overnight fast. Blood was collected in evacuated tubes containing no additives, allowed to clot, and centrifuged at 3000*g* for 10 minutes at room temperature to separate the serum. Blood samples for glycated hemoglobin (HbA_{1c}) were also collected in evacuated tubes containing no

additives. In accordance with the survey protocol, blood samples were rapidly transported (with no antioxidants) at −20°C by car (and airplane) to Mitsubishi Kagaku Bio-Clinical Laboratories Inc (Tokyo, Japan) and stored at −20°C until analysis.

2.3. Serum fatty acids

Serum fatty acid levels were analyzed at Mitsubishi Kagaku Bio-Clinical Laboratories Inc (Tokyo, Japan) within 70 days of sample collection, using the following procedure, which is similar to that described elsewhere [18]. Lipids were extracted from serum with chloroform and methanol under a nitrogen atmosphere and saponified with potassium hydroxide and ethanol. The lipids were transesterified to methyl esters of fatty acids with boron fluoride-methanol reagent, benzene, and methanol. The methyl esters were analyzed in a HP 6890 gas chromatograph (Agilent, Palo Alto, Calif) equipped with a flame ionization detector, using a capillary column (DB-WAX; 30 m × 0.32 mm internal diameter; 0.25 μg thickness; Agilent, Palo Alto, Calif). The identity of 24 individual fatty acid peaks was ascertained by comparing each peak's retention time to that of the retention times of fatty acids in synthetic standards of known fatty acid composition. The relative amount of each fatty acid (% of total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids. In-house quality-control procedures were conducted at Mitsubishi Kagaku Bio-Clinical Laboratories Inc. The mean value of fatty acid recovery during the serum extraction procedure was 75%. A commercially available standard material (pentadecanoic acid; Tokyo Chemical Industry Co Ltd, Tokyo, Japan) was used as an internal standard for fatty acid analysis. The coefficient of variations for the 24 fatty acids ranged from 2.6% to 18.3% (16:0, 3.9%; 18:0, 8.6%; 16:1n-7, 3.6%; 18:1n-9, 3.9%; 18:2n-6, 3.4%; 18:3n-6, 8.6%; 20:3n-6, 3.8%; 20:4n-6, 4.0%) with a median of 5.8%. Fatty acid composition was expressed as molecular percentage per milliliter of total serum.

2.4. Estimated activities of desaturase and elongase enzymes

Desaturase and elongase activities were estimated as the ratio of product to the precursor of individual fatty acids in serum (molecular percentage) according to the following: D9D = 16:1n-7/16:0 and 18:1n-9/18:0; D6D = 18:3n-6/18:2n-6; D5D = 20:4n-6/20:3n-6; and elongase = 18:0/16:0. From this point forward in the text, the ratios of 16:1n-7 to 16:0 and of 18:1n-9 to 18:0 will be referred to as D9D-16 and D9D-18, respectively.

2.5. Metabolic risk factors

Body height was measured to the nearest 0.1 cm with the subject standing without shoes. Body weight in light indoor clothes was measured to the nearest 0.1 kg. Body mass index (BMI) was calculated as body weight (kilogram) divided by the square of body height (square meter). Waist circumference was measured at the level of the umbilicus to the nearest 0.1 cm. The measurement was taken at the end of a normal expiration while the subject was standing erect with her arms at her side and feet together. Systolic and diastolic blood pressure was measured on the left arm with an automatic device (Omron model HEM-770A; Omron Health Care, Kyoto, Japan) after the subject had been sitting quietly for at least 3 minutes. A second measurement was carried out about 1 minute after the first, and the mean value of the two was used. The biochemical variables listed below were assayed at Mitsubishi Kagaku Bio-Clinical Laboratories Inc within 2 days of sample collection. Serum total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol, triacylglycerol, and glucose concentrations were measured by enzymatic assay methods. Serum insulin was determined by immunoradiometric assay. Glycated hemoglobin was measured by latex agglutination-turbidimetric immunoassay. In-house quality-control procedures for all assays were conducted at Mitsubishi Kagaku Bio-Clinical Laboratories Inc. Degree of insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) as follows: fasting glucose (mmol/L) × fasting insulin (mU/L)/22.5 [19].

2.6. Other variables

In a lifestyle questionnaire, the subject reported her residential area, which was grouped into 1 of 3 regions (region of Japan: north [Kanto, Hokkaido, and Tohoku], central [Tokai, Hokuriku, and Kinki], or south [Kyushu and Chugoku]). The residential areas were also grouped into 3 categories according to population size (size of residential area: city with population of at least 1 million, city with population of less than 1 million, or town and village). Current smoking status (yes or no) was self-reported in the questionnaire. According to information on whether the subject was currently menstruating, the date of the start of the last (current) menstruation, and the usual length of the menstrual cycle as reported in the questionnaire, as well as the date the lifestyle questionnaire was completed, the subjects were divided into 3 categories of menstrual cycle phase at the time of the study (menstrual [during menstrual flow], premenstrual [the week before the beginning of menstrual flow], or intermenstrual [remainder of cycle] phases). Physical activity was computed as the average metabolic equivalent hours per day [20] on the basis of the frequency and duration of 5 different activities (sleeping, high-intensity and moderate-intensity activities, walking, and sedentary activities) for the preceding month as reported in the questionnaire. Dietary habits during the preceding month were assessed using a validated, self-administered, comprehensive diet history questionnaire [21,22]. Estimates of dietary intake for a total of 150 food items, energy, and selected nutrients were calculated using an ad hoc computer algorithm for the diet history questionnaire [21], which was based on the Standard Tables of Food Composition in Japan [23].

2.7. Statistical analysis

Estimated activity of D9D-16, D9D-18, D6D, D5D, and elongase was examined in relation to 12 metabolic risk factors, such as BMI, waist circumference, systolic and diastolic blood pressures, cholesterol (total, HDL, and LDL), triacylglycerol, glucose, HbA_{1c}, insulin, and HOMA-IR. All statistical analyses were performed using SAS statistical software (version 8.2; SAS Institute Inc, Cary, NC). The β coefficients (and SE) were calculated for each of the estimated desaturase and elongase activities by linear regression analysis (using the Proc Reg procedure) with the respective metabolic risk factor as the dependent variable, with or without adjustment for the potential confounding variables. Confounding factors included region of Japan, size of residential area, current smoking, menstrual cycle phase at the time of the study, and physical activity. Because alcohol intake was extremely low (mean, 1.5 g/d), it was not considered as a confounding factor. Body mass index was added as a confounding factor in all analyses except that for BMI itself. Skewed data (ie, triacylglycerol, insulin, and HOMA-IR) were natural-log transformed. All reported P values are 2-tailed, and a P value of less than .05 was considered statistically significant.

3. Results

Subject characteristics are shown in Table 1. Overall, subjects were characterized by favorable metabolic risk factor profiles and a low smoking rate with a high consumption of fish. The percentage of subjects with abnormal values for metabolic risk factors [24-27] ranged from 0% to 12.0%. None of the estimated desaturase and elongase activities was significantly associated with current smoking, menstrual cycle phase at the time of the study, or physical activity (data not shown). Dietary and serum fatty acid compositions are shown in Table 2. The mean fatty acid contents of diet were characterized by a high proportion of oleic acid (18:1n-9), linoleic acid (18:2n-6), and palmitic acid (16:0), and a moderate proportion of stearic acid (18:0), α-linolenic acid (18:3n-3), and myristic acid (14:0), with nonnegligible proportion of docosahexaenoic acid (22:6n-3) and eicosapentaenoic acid (20:5n-3). The mean fatty acid profiles of total serum lipids were characterized by a high proportion of linoleic acid (18:2n-6), palmitic acid (16:0), and oleic acid (18:1n-9), and a moderate proportion of arachidonic acid (20:4n-6), stearic acid (18:0), docosahexaenoic acid (22:6n-3), palmitoleic acid (16:1n-7), and eicosapentaenoic acid (20:5n-3).

Table 3 shows the relations of estimated desaturase and elongase activities with metabolic risk factors. The activity of D9D-16 was significantly and positively associated with BMI, irrespective of adjustment for region of Japan, size of residential area, current smoking, and physical activity. In addition, that of D9D-16 showed a significant positive association with diastolic blood pressure, total and LDL cholesterol, and triacylglycerol and a negative association with HDL cholesterol, even after adjustment for not only the demographic and lifestyle factors but also BMI. Significant positive relations were also observed with waist circumference and systolic blood pressure, even after adjustment for the demographic and lifestyle factors, but these were not independent of BMI. Similar significant associations were

Table 1
Characteristics of subjects (n = 640)[a]

Variable	Value
Age (y)	19.7 ± 1.1
Body height (cm)	158.6 ± 5.4
Body weight (kg)	53.8 ± 7.5
BMI (kg/m^2)	21.4 ± 2.6
Waist circumference (cm)	72.5 ± 6.8
Systolic blood pressure (mm Hg)	106.0 ± 10.7
Diastolic blood pressure (mm Hg)	69.6 ± 8.1
Total cholesterol (mmol/L)	4.91 ± 0.84
HDL cholesterol (mmol/L)	1.82 ± 0.32
LDL cholesterol (mmol/L)	2.80 ± 0.72
Triacylglycerol (mmol/L)	0.63 (0.47, 0.82)
Glucose (mmol/L)	4.67 ± 0.35
HbA_{1c} (%)	4.82 ± 0.23
Insulin (mU/L)	7.30 (5.15, 9.70)
HOMA-IR	1.50 (1.05, 2.04)
Percentage of subjects with abnormal values for metabolic risk factors	
BMI ≥ 25 kg/m^2 [24]	8.6
Waist circumference ≥ 80 cm [24]	12.0
Systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg [25]	5.2
Total cholesterol ≥ 6.22 mmol/L (240 mg/dL) [25]	7.2
HDL cholesterol < 1.29 mmol/L (50 mg/dL) [25]	3.6
LDL cholesterol ≥ 4.14 mmol/L (160 mg/dL) [25]	4.8
Triacylglycerol ≥ 1.69 mmol/L (150 mg/dL) [25]	1.6
Glucose ≥ 6.1 mmol/L (110 mg/dL) [25]	0.2
HbA_{1c} ≥ 6.0% [26]	0
Insulin > 24 mU/L [26]	0.9
HOMA-IR ≥ 3.8 [27]	2.8
Current smoking	
No	98.1
Yes	1.9
Menstrual cycle phase at the time of the study	
Menstrual phase (during menstrual flow)	18.1
Premenstrual phase (the week before the beginning of menstrual flow)	29.4
Intermenstrual phase (remainder of cycle)	52.5
Physical activity (total metabolic equivalents h/d)	33.8 ± 2.8
Dietary intake	
Total energy (kJ/d)	7406 ± 2008
Total fat (g/d)	58.0 ± 22.4
Total fat (% of energy intake)	28.9 ± 5.3
Fish (g/d)	48.9 ± 33.4
Fish (g/4184 kJ of energy intake)	27.0 ± 14.4
Estimated activity of desaturase and elongase enzymes	
D9D-16 (16:1n-7/16:0)	0.08 ± 0.02
D9D-18 (18:1n-9/18:0)	3.23 ± 0.50
D6D (18:3n-6/18:2n-6)	0.007 ± 0.004
D5D (20:4n-6/20:3n-6)	7.01 ± 1.92
Elongase (18:0/16:0)	0.25 ± 0.03

[a] Values are presented as mean ± SD, median (interquartile range), or percentage of subjects.

Table 2
Dietary and serum fatty acid composition (n = 640)[a]

Variable	Diet (g/d)	Diet (mol% of total dietary fatty acids)	Serum (mol% of total serum lipids)
Saturated fatty acids	15.9 ± 6.8	34.5 ± 4.8	30.7 ± 1.4
12:0 (lauric acid)	0.6 ± 0.5	1.7 ± 1.2	0.2 ± 0.1
14:0 (myristic acid)	1.2 ± 0.7	3.1 ± 1.2	1.2 ± 0.3
16:0 (palmitic acid)	9.5 ± 3.8	20.9 ± 2.1	23.5 ± 1.0
18:0 (stearic acid)	4.3 ± 2.1	8.3 ± 1.7	5.9 ± 0.7
20:0 (arachidic acid)	0.19 ± 0.08	0.33 ± 0.05	0.04 ± 0.01
22:0 (behenic acid)	0.09 ± 0.04	0.15 ± 0.03	0.05 ± 0.01
24:0 (lignoceric acid)	0.04 ± 0.02	0.07 ± 0.02	0.04 ± 0.02
Monounsaturated fatty acids	20.2 ± 8.1	40.3 ± 2.3	21.2 ± 2.2
14:1n-5 (myristoleic acid)	0.09 ± 0.05	0.2 ± 0.1	0.1 ± 0.1
16:1n-7 (palmitoleic acid)	0.7 ± 0.3	1.5 ± 0.3	2.0 ± 0.4
18:1n-9 (oleic acid)	18.7 ± 7.5	37.4 ± 2.4	18.9 ± 2.0
20:1n-9 (eicosenoic acid)	0.4 ± 0.3	0.8 ± 0.3	0.11 ± 0.02
22:1n-9 (erucic acid)	0.2 ± 0.3	0.4 ± 0.4	0.02 ± 0.01
24:1n-9 (tetracosenoic acid)	0.03 ± 0.03	0.05 ± 0.03	0.08 ± 0.04
Polyunsaturated fatty acids	12.5 ± 4.7	25.1 ± 3.4	48.1 ± 2.9
n-3 polyunsaturated fatty acids	2.1 ± 0.9	4.2 ± 0.9	5.4 ± 1.6
18:3n-3 (α-linolenic acid)	1.6 ± 0.7	3.3 ± 0.6	0.7 ± 0.2
20:5n-3 (eicosapentaenoic acid)	0.2 ± 0.1	0.3 ± 0.2	1.4 ± 0.9
22:5n-3 (docosapentaenoic acid)	0.05 ± 0.04	0.08 ± 0.05	0.5 ± 0.1
22:6n-3 (docosahexaenoic acid)	0.3 ± 0.2	0.5 ± 0.3	2.9 ± 0.7
n-6 polyunsaturated fatty acids	10.4 ± 3.8	21.4 ± 2.8	42.6 ± 3.0
18:2n-6 (linoleic acid)	10.2 ± 3.8	21.0 ± 2.8	35.2 ± 2.9
18:3n-6 (gamma-linolenic acid)	0.004 ± 0.003	0.008 ± 0.005	0.2 ± 0.1
20:2n-6 (eicosadienoic acid)	0.05 ± 0.03	0.10 ± 0.04	0.18 ± 0.03
20:3n-6 (dihomo-γ-linoleic acid)	0.02 ± 0.01	0.05 ± 0.01	0.9 ± 0.2
20:4n-6 (arachidonic acid)	0.14 ± 0.06	0.26 ± 0.07	6.0 ± 1.0
22:4n-6 (docosatetraenoic acid)	0.006 ± 0.003	0.010 ± 0.004	0.10 ± 0.02
20:3n-9 (5-8-11 eicosatrienoic acid)	NA	NA	0.05 ± 0.01

NA indicates not available.
[a] Values are presented as mean ± SD.

observed for D9D-18, except for a significant positive relation with systolic blood pressure even after adjustment for BMI and no relation with total cholesterol.

The activity of D6D was significantly and positively associated with BMI after adjustment for the demographic and lifestyle factors. In addition, D6D activity showed a significant positive relation with systolic blood pressure, triacylglycerol, and HOMA-IR, even after adjustment for not only the demographic and lifestyle factors but also BMI. A significant positive relation was also observed with insulin, even after adjustment for the demographic and lifestyle factors, but these were not independent of BMI.

Conversely, the activity of D5D was significantly and negatively associated with BMI, regardless of adjustment for the demographic and lifestyle factors. In addition, D5D activity showed a significant negative association with systolic blood pressure, triacylglycerol, insulin, and HOMA-IR, regardless of adjustment for the demographic and lifestyle factors and BMI. Significant negative relationships for waist circumference, diastolic blood pressure, and glucose and significant positive relationship for HDL cholesterol were also observed, but these appeared to be explained by the demographic and lifestyle factors, BMI, or both.

Elongase activity was significantly and negatively associated with BMI, even after adjustment for the demographic and lifestyle factors. In addition, elongase activity showed a significant negative association with systolic and diastolic blood pressure and triacylglycerol, and a significant positive association with HDL cholesterol, even after adjustment for the demographic and lifestyle factors and BMI. A significant negative relation with waist circumference and LDL cholesterol was also observed, even after adjustment for the demographic and lifestyle factors, but this relation disappeared after adjustment for BMI.

4. Discussion

In this study of free-living young Japanese women, we found that increased estimates of D9D and D6D activity and decreased estimates of D5D and elongase activity were associated with adverse profiles of several metabolic risk factors. To our knowledge, this is the first study to examine the association between the estimated activities of enzymes involved in endogenous fatty acid synthesis and metabolic risk factors among young adult populations living in non-Western countries. Because the study population consisted of

Table 3
Association of estimated desaturase and elongase activities with metabolic risk factors (n = 640)[a]

	D9D-16 (16:1n-7/16:0)		D9D-18 (18:1n-9/18:0)		D6D (18:3n-6/18:2n-6)		D5D (20:4n-6/20:3n-6)		Elongase (18:0/16:0)	
	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*
BMI (kg/m^2)										
Model 1	0.39 ± 0.12	.0006	0.34 ± 0.10	.0009	0.21 ± 0.11	.06	−0.60 ± 0.10	<.0001	−0.24 ± 0.10	.015
Model 2	0.40 ± 0.12	.0006	0.33 ± 0.10	.001	0.23 ± 0.11	.036	−0.61 ± 0.10	<.0001	−0.22 ± 0.10	.025
Waist circumference (cm)										
Model 1	0.95 ± 0.31	.002	0.94 ± 0.27	.0005	0.38 ± 0.29	.19	−1.36 ± 0.27	<.0001	−0.77 ± 0.26	.003
Model 2	0.90 ± 0.30	.003	0.87 ± 0.26	.001	0.44 ± 0.29	.13	−1.44 ± 0.27	<.0001	−0.63 ± 0.26	.014
Model 3	0.11 ± 0.20	.58	0.23 ± 0.17	.19	−0.02 ± 0.19	.90	−0.21 ± 0.17	.22	−0.19 ± 0.17	.25
Systolic blood pressure (mm Hg)										
Model 1	1.11 ± 0.48	.021	2.09 ± 0.42	<.0001	1.62 ± 0.45	.0004	−1.98 ± 0.42	<.0001	−0.85 ± 0.37	.038
Model 2	1.25 ± 0.46	.007	2.05 ± 0.40	<.0001	1.31 ± 0.44	.003	−1.79 ± 0.40	<.0001	−1.11 ± 0.39	.005
Model 3	0.76 ± 0.44	.08	1.67 ± 0.38	<.0001	1.03 ± 0.42	.014	−1.06 ± 0.38	.007	−0.84 ± 0.37	.026
Diastolic blood pressure (mm Hg)										
Model 1	1.08 ± 0.36	.003	2.12 ± 0.31	<.0001	0.65 ± 0.35	.06	−1.11 ± 0.33	.0005	−1.01 ± 0.31	.001
Model 2	1.14 ± 0.36	.002	2.10 ± 0.30	<.0001	0.44 ± 0.34	.20	−0.96 ± 0.31	.002	−1.17 ± 0.30	.0001
Model 3	0.82 ± 0.35	.019	1.85 ± 0.30	<.0001	0.25 ± 0.33	.45	−0.46 ± 0.31	.14	−0.99 ± 0.29	.0009
Total cholesterol (mmol/L)										
Model 1	0.10 ± 0.04	.008	−0.01 ± 0.04	.71	0.01 ± 0.04	.77	0.06 ± 0.04	.12	0.01 ± 0.03	.87
Model 2	0.09 ± 0.04	.013	−0.02 ± 0.04	.64	0.01 ± 0.04	.85	0.06 ± 0.04	.12	0.00 ± 0.03	.94
Model 3	0.09 ± 0.04	.018	−0.02 ± 0.04	.56	0.00 ± 0.04	.91	0.06 ± 0.04	.07	0.01 ± 0.03	.87
HDL cholesterol (mmol/L)										
Model 1	−0.04 ± 0.01	.007	−0.12 ± 0.01	<.0001	−0.02 ± 0.01	.14	0.02 ± 0.01	.07	0.07 ± 0.01	<.0001
Model 2	−0.04 ± 0.01	.003	−0.12 ± 0.01	<.0001	−0.03 ± 0.01	.07	0.02 ± 0.01	.030	0.07 ± 0.01	<.0001
Model 3	−0.03 ± 0.01	.019	−0.12 ± 0.01	<.0001	−0.02 ± 0.01	.15	0.01 ± 0.01	.29	0.07 ± 0.01	<.0001
LDL cholesterol (mmol/L)										
Model 1	0.11 ± 0.03	.0006	0.09 ± 0.03	.002	0.01 ± 0.03	.83	0.01 ± 0.02	.67	−0.06 ± 0.03	.045
Model 2	0.11 ± 0.03	.001	0.09 ± 0.03	.002	0.01 ± 0.03	.74	0.01 ± 0.03	.82	−0.06 ± 0.03	.046
Model 3	0.09 ± 0.03	.005	0.08 ± 0.03	.008	0.00 ± 0.03	.98	0.04 ± 0.04	.25	−0.05 ± 0.03	.09
ln(triacylglycerol [mmol/L])										
Model 1	0.12 ± 0.02	<.0001	0.17 ± 0.02	<.0001	0.11 ± 0.02	<.0001	−0.12 ± 0.02	<.0001	−0.05 ± 0.02	.0006
Model 2	0.12 ± 0.02	<.0001	0.16 ± 0.02	<.0001	0.10 ± 0.02	<.0001	−0.12 ± 0.02	<.0001	−0.06 ± 0.02	<.0001
Model 3	0.12 ± 0.02	<.0001	0.16 ± 0.02	<.0001	0.10 ± 0.02	<.0001	−0.12 ± 0.02	<.0001	−0.06 ± 0.02	.0002
Glucose (mmol/L)										
Model 1	−0.01 ± 0.02	.65	0.00 ± 0.01	.84	0.03 ± 0.02	.08	−0.04 ± 0.02	.027	0.02 ± 0.01	.17
Model 2	−0.01 ± 0.02	.69	0.00 ± 0.01	.94	0.02 ± 0.02	.16	−0.02 ± 0.01	.054	0.01 ± 0.01	.30
Model 3	−0.01 ± 0.02	.63	0.00 ± 0.01	.99	0.02 ± 0.02	.17	−0.02 ± 0.01	.06	0.01 ± 0.01	.28
HbA_{1c} (%)										
Model 1	−0.01 ± 0.01	.20	−0.01 ± 0.01	.47	0.01 ± 0.01	.38	−0.00 ± 0.01	.65	0.01 ± 0.01	.45
Model 2	−0.01 ± 0.01	.21	−0.01 ± 0.01	.42	0.01 ± 0.01	.45	−0.00 ± 0.01	.75	0.01 ± 0.01	.46
Model 3	−0.01 ± 0.01	.20	−0.01 ± 0.01	.40	0.01 ± 0.01	.46	−0.00 ± 0.01	.76	0.01 ± 0.01	.45
ln(insulin [mU/L])										
Model 1	0.03 ± 0.03	.26	0.06 ± 0.03	.016	0.06 ± 0.02	.011	−0.12 ± 0.02	<.0001	−0.00 ± 0.02	.99
Model 2	0.03 ± 0.03	.27	0.05 ± 0.03	.024	0.06 ± 0.02	.014	−0.12 ± 0.02	.0001	−0.00 ± 0.02	.91
Model 3	0.00 ± 0.03	.97	0.03 ± 0.02	.18	0.05 ± 0.02	.06	−0.08 ± 0.02	.0001	0.01 ± 0.02	.54
ln(HOMA-IR)										
Model 1	0.03 ± 0.03	.31	0.06 ± 0.03	.017	0.07 ± 0.03	.008	−0.13 ± 0.02	<.0001	0.00 ± 0.02	.89
Model 2	0.03 ± 0.03	.31	0.05 ± 0.03	.027	0.07 ± 0.03	.011	−0.13 ± 0.02	<.0001	−0.00 ± 0.02	.99
Model 3	−0.00 ± 0.03	.98	0.03 ± 0.05	.19	0.05 ± 0.02	.045	−0.10 ± 0.02	<.0001	0.02 ± 0.02	.48

[a] β values are shown for a 1-SD increase in estimated desaturase and elongase activities. Model 1, crude model. Model 2, adjusted for region of Japan (north [Kanto, Hokkaido, and Tohoku], central [Tokai, Hokuriku, and Kinki], or south [Kyushu and Chugoku]), size of residential area (city with population of at least 1 million, city with population with less than 1 million, or town and village), current smoking (yes or no), menstrual cycle phase at the time of the study (menstrual [during menstrual flow], premenstrual [the week before the beginning of menstrual flow], or intermenstrual [remainder of cycle] phases), and physical activity (total metabolic equivalents h/d, continuous). Model 3, in addition, adjusted for BMI (kilogram per square meter, continuous).

generally healthy persons, the clinical relevance of our findings remains to be elucidated. Nevertheless, our results should provide valuable insight from a prevention perspective. The relatively healthy profiles of metabolic risk factors in these young, healthy, and lean women suggest that greater differences might be seen in other populations.

Only a limited number of observational studies of adult populations in Western countries have examined estimated desaturase and elongase activities in relation to metabolic risk factors. Estimated activity of D5D but not D9D-16 or D6D at 50 years of age was independently negatively associated with the development of metabolic syndrome

after 20 years in 654 Swedish men [3]. Estimated activity of D5D was also independently negatively related to fasting insulin in 27 patients with coronary heart disease (mean age, 58 years) and also in 13 healthy men (30 years) [4]. In middle-aged Swedish men and women (n = 554 and 295, respectively), estimated activity of D9D-16 but not D9D-18, D6D, or D5D was independently related to being overweight (BMI > 25 kg/m^2) [5]. Estimated activity of D9D-18 was also independently inversely correlated with insulin sensitivity as determined by hyperinsulinemic-euglycemic clamp in a German study of 98 healthy individuals (age not available) [6]. In addition, estimated activity of D9D-18 was independently associated with higher triacylglycerol in 411 Japanese, 418 Korean, and 251 Mongolian men and women aged 30 to 60 years [11]. A Swedish study of 381 men aged 69 to 73 years showed that estimated activity of D6D, but not D9D-18, D5D, or elongase, was independently associated with plasminogen activator inhibitor levels [7]. Among other studies, findings include a positive relation of estimated activity of D9D-18 to percentage of body fat and BMI, and a negative relation of estimated activity of D5D to fasting insulin, percentage of body fat, and BMI, and of elongase to percentage of body fat and BMI in 52 adult male Pima Indians [8]; a positive relation of estimated activity of D9D-16 and D6D, but not D9D-18, with glucose and insulin concentrations, and a negative association for estimated activity of D5D in 520 middle-aged Finnish adults with varying degree of glucose tolerance [9]; and a positive association between estimated activity of D9D and triacylglycerol (both D9D-16 and D9D-18), and a negative association between estimated activity of D9D and HDL cholesterol (D9D-18 only) in 173 white individuals aged at least 20 years [10]. Adjustment for confounding factors was not carried out in these studies. These results generally suggest that high estimates of D9D and D6D activity and low estimates of D5D and elongase activity are associated with unfavorable profiles of metabolic risk factors. The role of enzymes involved in endogenous fatty acid synthesis in developing metabolic disorder is not clear. Activity of desaturase and elongase enzymes may affect insulin action, an important pathogenetic factor of metabolic disorder, through its influence on membrane fluidity [4].

In the present study, estimated activity of D9D was independently positively associated with BMI, systolic (D9D-18 only) and diastolic blood pressures, total (D9D-16 only) and LDL cholesterol, and triacylglycerol and negatively associated with HDL cholesterol. Estimated activity of D6D also showed independent and positive associations with BMI, systolic blood pressure, triacylglycerol, and HOMA-IR. Conversely, estimated activity of D5D showed independent and negative associations with BMI, systolic blood pressure, triacylglycerol, insulin, and HOMA-IR. Estimated elongase activity was also independently associated negatively with BMI, systolic and diastolic blood pressures, and triacylglycerol and associated positively with HDL cholesterol. In the present study, mean daily intake of fish and eicosapentaenoic acid plus docosahexaenoic acid was 49 g and 0.44 g (0.22% of energy intake), respectively, whereas mean concentration of serum n-3 polyunsaturated fatty acid was 6.16% of total serum lipids. Although direct comparison cannot be made, these values are likely higher than those observed in the aforementioned Western studies, given the marked differences between Japanese and white adults observed in previous studies [15,16]. Despite such profound differences, our results of an association between estimated desaturase and elongase activities and metabolic risk factors are generally consistent with the previous Western studies.

Several limitations of our study warrant mention. First, we used the ratio between individual serum fatty acids as estimates of desaturase and elongase activities because direct measures of enzyme activities are not feasible in large epidemiological studies. Thus, conclusions about actual enzyme activity cannot be made, although estimated enzyme activities may be useful for understanding fatty acid synthesis patterns associated with metabolic risk factors. Second, although most published works have used fatty acids in serum cholesteryl esters [3,5,7,9] and plasma triacylglycerols [6], as well as skeletal muscle phospholipids [4,8] for estimating enzyme activities, we assessed fatty acid composition in total serum lipids. The main weakness with serum (or plasma) fatty acids is that they are noticeably influenced by the short-term fat intakes [28], the potential advantage being that they do reflect the total circulating fatty acids [29]. Considering that the difference in lipid fractions used results in somewhat different proportions of fatty acids (for example, n-3 fatty acids are high in phospholipids but low in triacylglycerols, and linoleic acid is high in cholesteryl esters) [30-32], more studies should be performed to check whether the ratios of total serum (or plasma) fatty acids are appropriate indicators of desaturase and elongase activities, although it should be noted that at least 2 previous studies did use total plasma lipids for estimating desaturase activities [10,11]. In the present study, there existed some paradoxical associations (eg, positive association of estimated activity of D9D-18 with LDL cholesterol but no association with total cholesterol and positive association of estimated activity of D6D with systolic blood pressure but no association with diastolic blood pressure), which might be at least partly explained using total serum fatty acids for estimation of enzyme activities. Nevertheless, the present results agree with the results from previous studies, which might provide some reassurance for the use of total serum fatty acids for estimating desaturase and elongase activities. Third, our subjects were selected female dietetic students, not a random sample of Japanese women. In addition, because of our recruitment procedure, the exact response rate was unknown, which might have produced recruitment bias. Thus, the present results cannot apply to the general Japanese population. Nevertheless, our population was, on average, comparable with a representative sample of Japanese women aged 20 to

29 years, at least with regard to several metabolic risk factors including BMI (20.9 kg/m^2), systolic blood pressure (108.8 mm Hg), diastolic blood pressure (67.0 mm Hg), total cholesterol (4.65 mmol/L), HDL cholesterol (1.78 mmol/L), and HbA_{1c} (4.91%), and dietary intake of energy (7042 kJ/d) and fat (55.1 g/d), although mean fish intake was low (68.0 g/d; data not available for other variables) [33]. Fourth, although our subjects were largely free from several lifestyle confounders such as smoking and alcohol drinking, we could not rule out residual confounding. Physical activity in particular was assessed relatively roughly from only 5 activities, which might not have been sufficient. Fifth, it is unknown whether desaturase and elongase activities are directly associated with metabolic risk factors because changes in them may be caused by changes in dietary fatty acid intake and serum fatty acid proportions that may in turn be directly associated with metabolic risk factors [1]. Thus, some of the present findings might be explained by dietary or serum fatty acid compositions rather than enzyme activities. Finally, the cross-sectional nature of the study does not permit the assessment of causality, owing to the uncertain temporality of the association. Changes in desaturase and elongase activities may be a consequence of changes in metabolic risk factors [1].

In conclusion, increased estimates of D9D and D6D activities and decreased estimates of D5D and elongase activities were associated with adverse profiles of several metabolic risk factors in a group of free-living young Japanese women. The physiopathologic relevance of these observations should be further determined.

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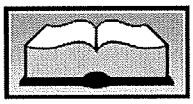

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Soft Drink Intake Is Associated with Diet Quality Even among Young Japanese Women with Low Soft Drink Intake

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ABSTRACT

Background Unsweetened traditional Japanese tea has long been the main beverage consumed in Japan, with soft drinks only recently forming a part of people's diets. Evidence suggests an association between soft drink intake and poor diet quality among youth in the United States. The association is not yet fully examined in the population with relatively low intake level of soft drinks such as the current Japanese population.

Objective To investigate the association of soft drink intake with dietary intake among young Japanese women.

Design A cross-sectional survey assessed dietary intake using a validated, self-administered, diet history questionnaire.

Subjects/setting Female dietetics students aged 18 to 20 years (n=3,931) in April 2005 in Japan.

Statistical analyses Multivariate linear regression analyses examined the relationship of soft drink intake with that of foods, beverages, energy, and nutrients.

Results Mean±standard deviation soft drink intake was 70.6±93.0 g/1,000 kcal. Soft drink intake was significantly associated positively with intake of confections, fat and oil, noodles, 100% vegetable and fruit juices, diet soft drinks, energy, and carbohydrates and negatively with intake of vegetables, fruits, pulses, fish and shellfish, rice, eggs, potatoes, milk, coffee and black tea, traditional Japanese tea, protein, dietary fiber, cholesterol, and most of the micronutrients examined.

Conclusions Not only among Western populations, but also among non-Western populations, soft drink intake may be an important factor to consider in evaluating overall dietary intake and diet quality.

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Some beverages are typically consumed in accompaniment with particular foods more frequently than with other foods (1-3). In the Unites States, sugar-sweetened carbonated beverages (ie, soft drinks) are consumed with snacks and with meals among adolescents (4-7), mainly at home (4,6). In particular, soft drink intake among youth in the United States has dramatically increased during the past 3 decades (4,6,8), and energy intake from soft drinks now comprises a significant part of the total energy intake of young people (4,5). Along with soft drink intake, intake of coffee, tea, and fruit juice was concomitantly increased while milk intake decreased (4). Accumulating evidence has linked soft drink intake and poor diet quality, such as higher intake of saturated fat and lower intake of key nutrients (7,9-12). Given these findings, the influence of soft drinks on diet quality is now a question of considerable public health concern (13).

The traditional Japanese diet consists of staple foods, mainly rice (30% of total energy intake) accompanied by other foods such as fish, vegetables, and soybean products (14). In the Japanese diet, unsweetened traditional Japanese teas, such as green tea and barley tea, have long

been the main beverage consumed at meals and between meals. On the other hand, drinking sugar-sweetened beverages at meals had not been a part of traditional Japanese diet. However, soft drinks became available with the Westernization of the diet following World War II. Because the mean daily intake of green tea, which accounts for the largest part of traditional Japanese tea intake, is still relatively high compared to other types of beverages, such as coffee, black tea, and milk (15), the intake of soft drinks may be lower in the Japanese than in the US population although no reliable data for soft drink intake are available. However, given that traditional Japanese beverages, including Japanese tea, are consumed without added sugar, in contrast to Western beverages, such as coffee and black tea, which are often consumed with added sugar, the effect of sugar-sweetened soft drinks on diet quality in the Japanese population may not be negligible even if the amount of soft drink intake is small.

Our study examined the association between soft drink intake and dietary intake among a young female Japanese population. Based on evidence from previous studies conducted in the United States (7,9-12) as well as implications from the traditional Japanese diet, the following hypothesis was formulated: Soft drink intake would be associated positively with intake of total energy; intake of food groups, including confections, fat and oil, meat, bread, eggs, coffee and tea, 100% fruit and vegetable juices, and diet soft drinks; and intake of nutrients, including carbohydrates, fat, and saturated fatty acids; and negatively with intake of food groups, including vegetables, fruits, pulses, fish and shellfish, rice, noodles, potatoes, milk products, milk, and traditional Japanese tea; and intake of nutrients, including protein, dietary fiber, cholesterol, sodium, potassium, calcium, magnesium, iron, vitamin A, vitamin C, vitamin D, vitamin E, thiamin, and riboflavin. A young population (women aged 18 to 20 years) was selected because this age group more vulnerable to poor diet quality (eg, higher fat intake) than other age groups (14).

METHODS

Subjects and Survey Procedure

Our study was a cross-sectional study based on a self-administered questionnaire survey among freshman students who enrolled in dietetics course in April 2005 (N=4,679). The survey was conducted at 54 universities, colleges, and technical schools in 33 of 47 prefectures in Japan. The study design and survey procedure are described in detail elsewhere (16). Briefly, subjects completed two questionnaires on dietary habits and other lifestyle items during the preceding month, in most institutions within 2 weeks after the course began. All questionnaires were checked at least once each by staff at the respective institution and 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 of Japan.

In total, 4,394 students (4,168 women and 226 men) answered two questionnaires (response rate 93.9%). For our analysis, female subjects aged 18 to 20 years (n=4,060) were selected. A new academic year in Japan starts in April. To minimize a possibility of dietary change or behavior change due to education related to diet and nutrition, the subjects who attended an institution at which the survey was conducted at the end of May (n=98) were excluded. Those with extremely low or high energy intake (<500 kcal/day or >4,000 kcal/day) (n=23), and those with missing information on the variables used (n=12) were also excluded. Some subjects were in more than one exclusion category, so the final analysis sample comprised 3,931 women. The basic characteristics of the 3,931 women are described in detail elsewhere (16).

Dietary Assessment

Dietary habits during the preceding month were assessed using a previously validated, self-administered diet history questionnaire (DHQ) (17-19). 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 semiquantitative portion size of 122 selected food and nonalcoholic beverage items, dietary supplement use, consumption frequency and semiquantitative portion size of 19 cereals usually consumed as staple foods (rice, bread, and noodles) and miso (fermented soybean paste) soup, and open-ended items for foods consumed regularly but not appearing in the DHQ.

The food and beverage items were selected as foods commonly consumed in Japan, mainly from a food list in the National Nutrition Survey of Japan, and standardized portion sizes were mainly derived from several books of recipes for Japanese dishes (17). For cereals and miso soup, consumption frequency was reported as the number per week (zero to seven) at each meal (breakfast, lunch, dinner, and snack). Portion size of rice and miso soup was estimated by asking the size of bowls usually used (five categories ranging from for child to large bowl) and the number (0.1 to 9.9) of bowls eaten at each meal. For bread and noodles, portion size was reported as the number (0.1 to 9.9) of standard units offered (eg, one slice for bread and one bowl typically consumed by one person at one meal for noodles). For other food items, consumption frequency was asked using eight categories, ranging from "two or more times a day" to "less than once a month." For beverage items, consumption frequency was asked using eight categories, ranging from "more than six times per day" to "less than once a week." For both food and beverage items, relative portion size was asked using five categories compared to a standard portion size indicated in words such as "a half" for apple and "one big leaf" for cabbage. The categories ranged from "very small (50% or less)" to "very large (50% larger or more)." Detailed descriptions of the methods used to calculate dietary intake and the validity of the DHQ have been published elsewhere (17-19).

Estimates of dietary intake for 148 food and beverage items, energy, and nutrients were calculated using an ad hoc computer algorithm for the DHQ based on the Standard Tables of Food Composition in Japan (20). Information on dietary supplements was not used due to the lack of a reliable composition table for dietary supplements in Japan. Also, because subjects responding to open-ended questions were few, data from the open-ended questions were not used.

Group	Items included in the group
Foods	
Confections	Rice crackers; snacks made from wheat flour; Japanese sweets with azuki beans; Japanese sweets without azuki beans; cakes; pastry; cookies and biscuits; chocolate; candies, caramels, and chewing gum; jellies; donuts; jam and marmalade; sugar used during cooking
Fat and oil	Butter, margarine, mayonnaise, salad dressing, oil used during cooking
Vegetables	Carrots, pumpkin, tomatoes, green peppers, broccoli, green leafy vegetables, salted pickled plums, other salted pickles, cabbage, cucumber, lettuce, Chinese cabbage, bean sprouts, radishes, onions, cauliflower, eggplant, burdock, lotus root, mushrooms (eg, shiitake, shimeji, and enoki), nori, seaweed (eg, wakame and hijiki)
Fruits	Raisins, canned fruits, oranges, bananas, apples, strawberries, grapes, peaches, pears, persimmons, kiwi fruits, melons, watermelons
Pulses	Tofu, tofu products, natto, boiled beans (eg, azuki, soy, and black beans), miso as seasoning, miso in miso soup, peanuts, other nuts
Fish and shellfish	Dried fish, small fish with bones, canned tuna, eel, white meat fish, blue-back fish, red meat fish, fish paste, shrimp, squid and octopus, oysters, other shellfish, fish eggs (eg, salmon roe and cod roe), boiled small fish and seaweeds seasoned with soy sauce, salted fish intestine
Meat	Ground beef and pork, chicken, pork, beef, liver, ham and sausages, bacon
Rice	White rice, white rice mixed with barley, white rice with rice germ, 50% polished rice, 70% polished rice, brown rice
Bread	White bread, butter roll, croissant, pizza, Japanese-style pancake, pancake, cornflakes
Noodles	Japanese noodle (eg, buckwheat and Japanese wheat noodle), instant noodle, Chinese noodle, pasta
Eggs	Eggs
Potatoes	Potato chips, french fries, potatoes, sweet potatoes, yam and taros, konnyaku (devil's tongue jelly)
Milk products	Cottage cheese, other cheese, sugar-sweetened yogurt, natural yogurt, low-fat yogurt, coffee cream, ice cream
Beverages	
Milk	Whole milk, low-fat milk, skim milk
Coffee and black tea	Unsweetened coffee, unsweetened black tea
100% vegetable and fruit juices	Vegetable juice, tomato juice, 100% fruit juice
Soft drinks	Regular or nondiet soft drinks, non–100% fruit juice, sugar-sweetened coffee, sugar-sweetened black tea, cocoa, lactic acid drinks, energy drinks
Diet soft drinks	Non–energy-containing soft drinks or diet soda
Traditional Japanese tea	Nonfermented (eg, green tea and barley tea) and semifermented types of tea (eg, oolong tea)

Figure. Definitions of food and beverage groups used to categorize dietary intake by female Japanese dietetics students aged 18 to 20 years. Foods and beverages that did not belong to a food group (eg, ketchup, nonoil salad dressing, soy sauce, roux for curry and chowder, corn soup, Chinese soup, soup in noodle soup, soup in miso soup, energy bars, artificial sweeteners, water, table salt, and salt used during cooking) and alcohol (eg, beer, sake, distilled liquor, cocktails, whisky, and wine) because intake was extremely low were not included in the analyses. Intake of coffee, black tea, and added sugar for coffee and black tea was asked as three separate questions in the 148 items. Intake of coffee and tea of the subjects who responded that they did not use sugar for coffee and tea was considered as unsweetened coffee and unsweetened black tea and then, categorized into the coffee and black tea group. Intake of coffee and black tea of the subjects who responded that they used sugar for coffee and black tea was considered as sugar-sweetened coffee and sugar-sweetened black tea and then, categorized into the soft drinks group. Thus, the total number of items in the Figure and footnotes are 149 items.

Definitions of food and beverage groups used in our study is shown in the Figure. Soft drinks included the following items: regular or nondiet soft drinks, non–100% fruit juices, sugar-sweetened coffee, sugar-sweetened black tea, cocoa, lactic acid drinks, and energy drinks. Diet soft drinks included non–energy-containing soft drinks or diet soda. Intake of coffee, black tea and added sugar for coffee and black tea was asked as three separate questions in the 148 items. Intake of coffee and tea of the subjects who responded that they did not use sugar for coffee and tea was considered as unsweetened coffee and unsweetened black tea and then, categorized into coffee and black tea group. Intake of coffee and black tea of the subjects who responded that they used sugar for coffee and black tea was considered as sugar-sweetened coffee and sugar-sweetened black tea and then, categorized into the soft drinks group. Thus, the total number of items in the Figure and footnotes are 149 items. Traditional Japanese tea intake was assessed by one question that included nonfermented (ie, green tea and barley tea) and semifermented types of tea (ie, oolong tea). Milk was considered one of the beverages so "milk" and "milk products" were categorized and analyzed separately. Foods and beverages that did not belong to defined groups were not used in the analyses. Alcohol intake was not used because of an extremely low mean intake (0.8 g/day) in this population (16).

The DHQ has been developed to rank individuals by nutrient and food intake as well as to obtain the absolute value. In a previous validation study among 47 women,