Table 4
Hazard ratio of death from ischemic stroke according to quartiles of dietary GI, energy-adjusted dietary GL, total carbohydrate intake, and rice intake among 12 561 men and 15 301 women in the Takayama study, Japan

	Men					Women					
	Quartile				*P* for trend	Quartile				*P* for trend	
	1	2	3	4		1	2	3	4		
GI											
No. of cases	15	16	16	13		6	15	13	32		
Age adjusted	1	1.00 (0.50-2.03)	1.22 (0.60-2.46)	0.91 (0.43-1.92)	.96	1	1.67 (0.64-4.31)	1.18 (0.44-3.12)	2.45 (1.01-5.92)	.03	
P interaction with sex[a]											.29
Energy-adjusted GL											
No. of cases	20	13	13	14		8	16	23	19		
Age adjusted	1	0.56 (0.28-1.12)	0.77 (0.38-1.55)	0.92 (0.47-1.83)	.77	1	1.42 (0.61-3.32)	1.63 (0.72-3.66)	1.59 (0.70-3.65)	.27	
P interaction with sex[a]											.73
Carbohydrate intake											
No. of cases	16	13	16	15		15	9	20	22		
Age adjusted	1	0.62 (0.30-1.30)	0.90 (0.45-1.80)	0.91 (0.45-1.85)	.97	1	0.43 (0.19-0.98)	0.80 (0.41-1.57)	0.86 (0.44-1.65)	.85	
P interaction with sex[a]											.78
Rice intake											
No. of cases	18	21	5	16		7	18	25	16		
Age adjusted	1	0.97 (0.51-1.82)	0.52 (0.19-1.41)	1.21 (0.61-2.37)	.97	1	1.53 (0.64-3.68)	1.14 (0.49-2.67)	1.67 (0.69-4.07)	.39	
P interaction with sex[a]											.37

[a] Adjusted for age, sex, and the single dietary factor of the interaction term.

stroke than men. That may also support that a significant risk increase of stroke with high dietary GI was only observed among women in the current study. Previously, the intake of carbohydrates was generally not found to predict the risk of diabetes [20-28]. The studies may support the findings of the current study because no clear association was observed between carbohydrate intake and the risk of total stroke.

Dietary GI may have influence on factors other than diabetes, such as others that lead to the increased risk of stroke. It was reported that low-GI diets increased body fat loss among overweight or obese young adults in an intervention study, and progression of atherosclerosis was accelerated with high carbohydrate intake from high-GI sources among women with coronary heart disease in a 3-year prospective study [30,31]. These results may be supported by the studies that indicated the inflammatory reactions with hyperglycemia [32,33]. In the current study, however, subjects with high dietary GI were likely to consume low level of dietary fat; and the effect of dietary GI on lipid profile, if it existed, could be overridden.

Findings from previous prospective cohort studies implied that dietary GL was positively associated with the risk of stroke [3-5], whereas the association between dietary GL and the risk of total stroke was not clear in the current study. This could be attributed to the fact that the subjects in the current study population were relatively slim. Two of the studies found associations between the dietary GL and stroke among overweight women but not among those who were not overweight [3,4]. Alternatively, our results may indicate that, although large amounts of carbohydrates were regularly consumed among Japanese population, the quality of carbohydrates (represented by dietary GI) is more important than its quantity (partially represented by dietary GL).

Differences in the association between dietary GI and stroke subtypes were not clear in the current study. Among women, the risk of mortality from ischemic stroke was increased among the subjects with the highest level of dietary GI; and a similar pattern of risk increase for hemorrhagic stroke was also observed, although the association was not significant. Previous studies consistently reported that diabetes increased the risk of ischemic stroke [23,24,28], whereas the risk of hemorrhagic stroke does not seem to increase among people with diabetes [7,24,34]. However, in the current study, the dietary GL and rice intake significantly increased the risk of hemorrhagic stroke; but the associations were not significant with the risk of ischemic stroke among women in the current study. The large consumption of rice and the dietary GL may have been linked to the Japanese-style diet [35]. It was reported in the Honolulu Heart Program that a Western-type diet, compared with an Oriental-style diet, tended to be inversely associated with thromboembolic and hemorrhagic stroke. However, a recent cohort study in Japan reported that the Japanese dietary pattern was associated with a decreased risk of total stroke mortality [36]. The dietary GI sourcing from food items other than rice was not associated with the risk of total stroke or hemorrhagic stroke in the current study, and the inverse association was observed with the risk of ischemic stroke among men. Nonetheless, there is still a possibility that

Table 5
Hazard ratio of death from stroke according to quartiles of dietary GI, energy-adjusted dietary GL, total carbohydrate intake, and rice intake among 6970 men and 9747 women whose BMI was less than 23 and 4886 men and 4698 women whose BMI was 23 or over in Takayama study, Japan

	Men						Women					
	Quartile				*P* for trend	*P* for interaction with BMI[a]	Quartile				*P* for trend	*P* for interaction with BMI[a]
	1	2	3	4			1	2	3	4		
GI												
BMI <23 kg/m^2												
No. of cases	19	17	20	17			5	19	21	36		
Age-adjusted HR	1	0.84 (0.44-1.62)	1.09 (0.58-2.05)	0.93 (0.49-1.80)	.99		1	2.92 (1.09-7.84)	2.55 (0.96-6.81)	3.86 (1.51-9.90)	.01	
BMI ≥23 kg/m^2												
No. of cases	8	8	4	5			6	7	8	8		
Age-adjusted HR	1	1.21 (0.45-3.23)	0.68 (0.21-2.26)	0.82 (0.27-2.50)	.57		1	1.00 (0.33-2.97)	1.06 (0.36-3.08)	0.94 (0.32-2.76)	.94	
						.94						.21
Energy-adjusted GL												
BMI <23 kg/m^2												
No. of cases	23	17	16	17			9	21	26	25		
Age-adjusted HR	1	0.65 (0.35-1.22)	0.79 (0.42-1.49)	0.90 (0.48-1.69)	.71		1	1.86 (0.85-4.06)	1.87 (0.87-4.01)	1.92 (0.90-4.13)	.15	
BMI ≥23 kg/m^2												
No. of cases	11	3	4	7			6	6	9	8		
Age-adjusted HR	1	0.27 (0.08-0.98)	0.48 (0.15-1.50)	0.85 (0.33-2.20)	.56		1	0.81 (0.26-2.52)	1.17 (0.42-3.31)	1.16 (0.40-3.35)	.64	
						.46						.73
Carbohydrate intake												
BMI <23 kg/m^2												
No. of cases	18	19	17	19			17	14	23	27		
Age-adjusted HR	1	0.96 (0.51-1.84)	0.94 (0.48-1.81)	1.14 (0.60-2.17)	.90		1	0.59 (0.29-1.20)	0.87 (0.46-1.62)	0.88 (0.48-1.62)	.93	
BMI ≥23 kg/m^2												
No. of cases	9	3	4	9			7	6	5	11		
Age-adjusted HR	1	0.27 (0.07-0.99)	0.40 (0.12-1.30)	0.96 (0.38-2.41)	.90		1	0.84 (0.28-2.49)	0.59 (0.19-1.86)	1.27 (0.49-3.28)	.64	
						.70						.65
Rice intake												
BMI <23 kg/m^2												
No. of cases	21	26	10	16			7	24	29	21		
Age-adjusted HR	1	1.09 (0.62-1.94)	0.67 (0.31-1.42)	0.87 (0.45-1.67)	.44		1	2.02 (0.87-4.72)	1.48 (0.64-3.42)	2.10 (0.89-4.94)	.22	
BMI ≥23 kg/m^2												
No. of cases	8	8	2	7			7	5	9	8		
Age-adjusted HR	1	0.91 (0.34-2.44)	0.50 (0.10-2.41)	1.10 (0.40-3.05)	.93		1	0.56 (0.18-1.78)	0.79 (0.29-2.14)	0.98 (0.36-2.73)	.77	
						.45						.87

[a] Adjusted for age, BMI, and the single dietary factor of the interaction term.

nutrients or food items typical in Japanese-style diet could have played a role as confounders in the association between the dietary variables and stroke or its subtypes. Further evaluation of the consumption of rice in relation to risk of stroke and its subtypes in a larger study is warranted.

The dietary GI may have been associated with risk factors of stroke subtypes, other than diabetes. Several studies reported that a low dietary GI was associated with a high concentration of serum high-density lipoprotein cholesterol [37,38]. Low serum cholesterol levels with a condition of high blood pressure are reported risk factors of hemorrhagic stroke [39,40]. If the prevalence of uncontrolled high blood pressure was high in the group with a high dietary GI, GL, or rice consumption, such a condition with a lowered level of high-density lipoprotein cholesterol might explain the increased the risk of hemorrhagic stroke observed among women.

In the current study, stratified analysis by BMI showed that dietary GI and dietary GL were significantly associated with increased risk of death from total stroke among women with BMI less than 23. The interaction terms between BMI and the dietary factors were not significant. The results contradicted previous studies reporting the association between dietary GL and total stroke, or the association between dietary GL and cardiovascular disease among overweight or obese women [3,4]. There were a limited number of events available in the current study, and they may be too few to draw a conclusion after the stratification.

This study has several limitations. The FFQ in this study was not specifically designed to derive dietary GI values, as in many other studies; and thus, no validation data for the estimation of the dietary GI or GL exist. The data we collected for food and nutrient intake and estimated dietary GL may have been overestimated by the FFQ. However, our questionnaire was designed to measure the relative intake of food and nutrients rather than the absolute value. Correlation coefficients between the value from the FFQ and that from the food record for carbohydrate intake were relatively low; and attenuation of association, if the association existed, may have occurred. It was suggested that the correlation needs to be at least 0.3 or 0.4 to detect associations between diet and disease [41], and our FFQ may have minimal validity for the assessment of carbohydrate intake. The diagnoses of outcome were based on death certificates; and the possibility of misdiagnosis, especially for the subtypes of stroke, cannot be ruled out for the findings of the current study. High dissemination of computerized tomography in Japan, however, may warrant sufficient accuracy for the use of death certificate in the current study [42,43]. The information in deaths was not able to be updated after 1999, and the number of deaths from the subtypes of stroke was not enough to control for the potential risk factors. The impact of initial exclusion of the subjects on the study results is unknown; but the fairly high participation rates reduce concern of bias from nonparticipation, and its systematic effect, if it exists, may be minimal. We repeatedly performed statistical tests, so the results should be interpreted with caution.

5. Summary

In conclusion, we found a positive relationship between the dietary GI and risk of mortality from stroke among women in a community-based cohort in Japan, where a high risk of stroke is still observed. The results of the study also suggest that the risk of mortality from ischemic stroke is increased with increased level of the dietary GI among women, and a positive trend is also suggested between dietary GL and mortality from hemorrhagic stroke among women. Conducting further studies that precisely investigate the biomarkers for glucose metabolism in relation to the dietary GI and the risk of stroke would be desirable, and further investigation of the risk factors of stroke subtypes in response to the dietary GI with a specific focus on male-female differences is recommended.

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Review Article

Factors to Consider in the Association Between Soy Isoflavone Intake and Breast Cancer Risk

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ABSTRACT

It has been suggested that soy isoflavones have protective effects against breast cancer. However, data from epidemiological studies are not conclusive. A recent meta-analysis showed that soy intake was inversely associated with breast cancer risk in Asian but not Western populations, which indicates that protection against breast cancer may require that women consume levels of soy typical in Asian diets. In addition to the amount of soy isoflavones consumed, the form and food source of isoflavones, timing of isoflavone exposure, estrogen receptor status of tumors, and equol-producer status and hormonal profile of individuals may modify the association between soy isoflavone intake and the risk of breast cancer. These factors might explain the heterogeneity of results from studies. This present report contrasts background data from Japanese and Western women to identify the potential modifying of these factors.

Key words: breast cancer; soy isoflavones; review

INTRODUCTION

There has been much interest in the potentially protective role of soy in breast cancer development. Soybeans are the main source of isoflavones, which are classified as phytoestrogens. Laboratory data show that isoflavones have a wide range of biological actions. First, they have an affinity for estrogen receptors in vitro.[1] Thus, they may act as antiestrogens by competing for the binding sites of estrogen receptors. Isoflavones also inhibit the activity of key enzymes that convert androgens to estrogens.[2] In addition, isoflavones have been shown to be anti-proliferative,[3] proapoptic,[4] anti-angiogenic,[5] anti-oxidative,[6] and anti-inflammatory.[7] Taken together, these findings indicate that soy isoflavone may be a potent agent for preventing breast cancer. However, some data suggest that isoflavones promote breast cancer. Indeed, it has been shown that genistein stimulates the growth of estrogen-sensitive breast cancer cells in ovariectomized mice.[8,9] Because of the apparent complexity of the relationships between isoflavones and breast cancer in laboratory studies, data from human studies are particularly important. However, current data from epidemiological studies are not conclusive. There is some epidemiological evidence of an inverse association between isoflavone and breast cancer, but it is inconsistent.[10] Thus, no consensus has emerged regarding the preventive aspects of isoflavones.

Recently, Wu et al conducted 2 separate meta-analyses of studies carried out in Asian and Western populations.[11] A meta-analysis of 8 studies showed that Asian women consuming the highest amount (≥20 mg/day) of dietary isoflavones had a 29% reduction in breast cancer risk, as compared with those with low consumption (<5 mg/day) of isoflavones. An approximately 50% reduction in risk was reported in a prospective study of Japanese women.[12] In contrast, a meta-analysis of 11 studies of women eating Western diets found no association between isoflavone intake and breast cancer risk. In that analysis, the median for the highest intake was 0.8 mg/day. Therefore, women may need to consume the amount of soy in Asian diets to gain protection against breast cancer. Alternatively, the protective effects may only be present when exposure to soy occurs early in life. Differences in (1) the form and source of isoflavones, (2) the biological response to isoflavones among ethnic groups, and (3) the interactions with individuals' hormonal profiles may also explain the heterogeneity of the findings. This present report attempts to address these issues by contrasting background data from Japanese and Western women.

SOY ISOFLAVONE INTAKE IN JAPAN

The incidence rate of breast cancer is historically much lower in Japan than in Western countries. One possible explanation

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for this is that Japanese consume much more soy foods containing isoflavones. The fact that the incidence rate of breast cancer among Japanese immigrants rises as the length of time in the host country increases[13,14] suggests that lifestyle changes, including a change in dietary isoflavone intake, may play a role. According to the Cancer Incidence in Five Continents Vol IX,[15] the incidence rate for breast cancer in 1998–2002 was 32.0 per 100 000 among Japanese women in Osaka, Japan; 105.6 per 100 000 among white women in Hawaii; and 107.5 per 100 000 among Japanese women in Hawaii.

The nutritional status of Japanese has been evaluated annually by the National Nutritional Survey (NNS)[16] for more than 50 years. About 20 000 individuals from 6000 households in a randomly selected 300 districts are invited to participate in this survey every year. Foods and beverages consumed by a family are weighted and recorded for 3 consecutive days (1-day records have been used since 1995). According to the NNS reports, the mean intake of soy products was 67.2 g/day in 1975 and 57.7 g/day in 2005. Recently, the Food Safety Commission in Japan estimated isoflavone intake (expressed as aglycone) among Japanese, using the 2002 NNS data (response rate unknown). The median and the 95th percentile isoflavone intakes were 18 and 70 mg/day, respectively. Mean estimates of daily consumption reported in other studies in Japan ranged from 26 to 54 mg.[17] A database of the isoflavone content of Japanese foods, based on a validated direct measurement method, was first reported by Kimira et al.[18] In a report from the Takayama study—a prospective study of residents in Takayama city, Gifu, Japan[19]—the mean estimate, based on a validated food frequency questionnaire, was 19.6 mg per 1000 kcal among 15 724 female residents aged 35 years or older (response rate, 85.3%).[20] Daily intake of isoflavones is estimated to be between 0.5 and 3 mg in the United States[21,22] and less than 2 mg in European countries.[23] In a recent study of 8809 adults from the National Health and Nutrition Examination Survey (NHANES), the mean isoflavone intake, based on one 24-hr diet recall, was 1.2 mg.[24] Changing dietary habits have been reported in migrant populations. For example, soy intake among Japanese-Americans appears to be higher than that of US whites and lower than that of Japanese living in Japan. Takata et al[25] reported that mean isoflavone intake, estimated from a food frequency questionnaire, was 11.6, 7.2, and 3.5 mg per 1000 kcal in Japanese women in Gifu, and Japanese and white women living in Hawaii, respectively. According to the 2002 NNS data in Japan, isoflavone intake was associated with age. Even among adults, younger age groups tended to consume less isoflavones: the reported mean daily intake of isoflavones was 19.4, 21.5, 23.5, 27.4, 33.6, and 29.6 mg per day for adults aged 20–29, 30–39, 40–49, 50–59, 60–69, and 70+ years, respectively. Soy isoflavone intake will likely decrease among Japanese in the future.

Biomarkers are also useful in estimating exposure to soy isoflavones. Yamamoto et al[26] collected 24-hour urine and serum samples from Japanese women enrolled in the Japan Public Health Center-based (JPHC) prospective study. The mean levels for daidzein and genistein were 17.0 (standard deviation [SD], 15.3) and 14.2 (14.1) μmol/day, respectively, in urine. The corresponding values in plasma were 119.9 (135.8) and 475.3 (510.4) nmol/L, respectively. These values were reasonably correlated with isoflavone intake estimated from food-frequency questionnaires or diet records (r = 0.22–0.48). Similar values in the urine, plasma, and serum of Japanese women have been reported in other studies.[27–29] Even in spot urine samples, isoflavone levels were well correlated with intakes estimated from diet records (r = 0.30 for daidzein and 0.27 for genistein).[30] In a study of 59 women aged 20 to 45 years, the median values were 4.0 and 3.2 nmol/mg creatinine for daidzein and for genistein, respectively, in first morning urine specimens.[31] Another study of 419 women reported that the mean daidzein level was 12.8 nmol/mg creatinine in spot urine samples collected at approximately 2:00 PM.[32] Among Western populations, reported urinary isoflavone levels have been much lower. The geometric mean levels were 0.27 and 0.08 nmol/mg creatinine for daidzein and genistein, respectively, in urine specimens of participants in the NHANES between 1999 and 2000.[33] In a study of UK women, the geometric means for daidzein and genistein were 0.55 and 0.28 nmol/mg creatinine, respectively, in spot urine, and 7.9 and 15.2 μmol/L, respectively, in serum.[34]

Sources of isoflavones also differ between Japanese and Western populations. The intake of traditional soy-based foods is high in Japan. Based on data from the Takayama study, the most common soy foods are tofu, miso (soybean paste), soybeans, fried tofu, natto (fermented soybeans), and soymilk. The fermented soy foods (miso and natto) accounted for about 40% of total isoflavone intake. In Western populations, the consumption of traditional soy foods is substantially lower, and a portion of total isoflavone consumption is derived from soy protein and soy flour added to a variety of foods.[22] Such uses are increasing.[35] Recently, new isoflavone datasets for non-soy based foods have been developed to estimate isoflavone intake more accurately. Horn-Ross et al[22] assessed the isoflavone intake in 447 non-Asian women in the San Francisco Bay area, using a new database. Mean intakes of genistein and daidzein were estimated to be 1.5 and 1.3 mg/day, respectively. Major sources were tofu, doughnuts, soymilk, white bread, and canned tuna. Interestingly, doughnuts accounted for about 20% of the average of daily intake of genistein and 15% of daidzein intake. It is likely that Japanese women are also consuming isoflavones from such "hidden" sources of soy. Using several databases of non-soy foods, such as cereals, eggs, dairy foods, meats, fish, nuts, and vegetables,[21,36–39] and assuming that processed meats and fish contain 2% soy protein by weight, isoflavone intake from

these non-soy foods was estimated to be approximately 0.68 mg per 1000 kcal among the women in the Takayama study (unpublished data). This value is substantially lower than the isoflavone estimate from soy foods (19.6 mg per 1000 kcal).

In fermented soy products, like natto or tempeh, aglycones are the principal form of isoflavone, whereas in unfermented soy products, like soy milk or soy supplements, the glucoside form is predominant.[39] Knowing the bioavailability of the different isoflavone forms is thus fundamental. Watanabe et al conducted a kinetic study of Japanese men after ingestion of 60 grams baked soybean powder (*kinako*).[40] Differences in the form of isoflavone (aglycone vs glucoside) are believed to alter isoflavone pharmacokinetics and, hence, the association between soy intake and breast cancer risk. However, there is no consensus among studies on the bioavailability of aglycone vs glucoside.[41] In addition to isoflavone form, the matrix in which it is delivered is likely to have an effect on pharmacokinetics. It is also possible that an individual's background diet may modify the association of isoflavone intake with breast cancer risk by affecting the pharmacokinetics of metabolism of isoflavones. In addition, frequency of ingestion may affect bioavailability of isoflavones.[41] An optimum steady-state serum isoflavone level would be expected from consuming relatively small doses of soy throughout the day, as opposed to a single dose at one time. Indeed, Gardner et al[42] observed that plasma isoflavone concentrations remained constant when soy foods were consumed at breakfast, lunch, and dinner.

Metabolism of isoflavone can vary greatly between individuals, even when they consume an identical amount of isoflavone-containing food.[43] Equol, a metabolite of daidzein, has been identified in the urine and blood samples of some but not all humans. Intestinal bacteria play an essential role in daidzein metabolism. Several candidate bacteria for daidzein metabolism, including one reported by Ueno et al,[44] have been suggested, but the human intestinal bacteria responsible for daidzein metabolism are yet to be identified.[45] Equol binds with greater affinity to estrogen receptors than its parent compound daidzein,[46] and it is superior to all other isoflavones in its antioxidant activity,[47] which suggests that its overall potency is greater. The prevalence of equol producers appears to be higher in Japanese than in whites,[27,48,49] which might explain the additional benefits conferred on Japanese women in terms of reduced breast cancer risk.

EARLY EXPOSURE TO SOY

In vivo studies have consistently shown that prepubertal or pubertal exposure to genistein reduces the incidence and/or multiplicity of chemically induced mammary tumors in experimental animals,[50] possibly because of alterations in gene expression and morphological changes in the mammary glands. The details have been described elsewhere.[51] To this author's knowledge, 3 case-control studies have examined the association between soy intake during childhood or adolescence and the risk of breast cancer among Asian women.[52–54] All reported a significant inverse association. Another case-control study of Canadian women also observed a significant inverse association between adolescent isoflavone intake and breast cancer risk.[55] A recent prospective study of Chinese women confirmed that a high intake of soy foods during adolescence was associated with a reduced risk of breast cancer.[56] Unfortunately, there has been no such study among Japanese women.

Perinatal factors are also thought to influence subsequent breast cancer development. Trichopoulous hypothesized that the developing breast is influenced by the fetal environment, particularly variations in hormone concentrations, which could mediate subsequent breast cancer development.[57] Some epidemiological studies observed associations between pre- and perinatal characteristics, such as birth order and birth weight, and subsequent risk of breast cancer.[58] There has been no study assessing the association between maternal soy intake during pregnancy and the risk of breast cancer in offspring; however, by altering the estrogen environment, soy exposure in utero may affect the subsequent risk of breast cancer.

In utero soy exposure occurs among Japanese. Adlercreutz et al[59] reported that in 7 Japanese women at delivery the mean levels of genistein and daidzein were 83.9 and 45.5 nmol/L, respectively, in maternal plasma, and 165 and 58.8 nmol/L, respectively, in umbilical cord plasma. They observed high correlations between levels in maternal and umbilical plasma ($r = 0.34$ for genistein, $r = 0.44$ for daidzein, and $r = 0.99$ for equol; $n = 7$). A study by the present author, which included 194 pregnant women, supported the hypothesis that isoflavone can be transferred from mother to fetus.[30] In that study, we also included an assessment of maternal dietary intake of soy during pregnancy and measurements of estrogen levels in maternal and umbilical cord blood; the mean soy isoflavone intake was 21.7 mg per day. The geometric mean levels for serum genistein and daidzein were 116.5 and 50.2 nmol/L, respectively, in maternal blood at delivery, and 126.9 and 38.6 nmol/L, respectively, in umbilical cord blood. Estradiol level in umbilical cord blood was unrelated to isoflavone levels in both maternal and umbilical cord blood.

Infants in Japan are weaned on to soy products between 6 and 12 months of age, after which they continue to receive isoflavone-containing foods indefinitely; tofu and miso soup are common baby foods. Based on intake frequencies of tofu, miso soup, natto, soybean flour, and soymilk reported by 288 mothers, 6-month-old Japanese infants consumed about 3.1 mg of soy isoflavone per day (unpublished data). Setchell et al[60] reported that soy-based formulas contained approximately 32 to 47 μg/ml of isoflavone, indicating that a 4-month old infant fed soy formula would consume 28 to 47 mg of isoflavones per day. In their study, the mean plasma

concentrations of genistein and daidzein were 2530 and 1160 nmol/L, respectively, in 7 infants fed soy-based formulas. An isoflavone intake of 3 mg for Japanese infants is not low, if we take into account the body size of babies; however, it would be much lower than that available from a soy-based formula. Badger et al[61] reported substantial differences between exposure to soy between Japanese and Americans. If these differences are real, then Japanese fetuses would be exposed to the same high isoflavone levels that were present in maternal circulation. Serum isoflavone levels among Japanese infants would then be expected to decline at birth and remain low, until soy foods were introduced as baby food. Thereafter, the level would increase to the usual adult levels as a result of habitual intake. American infants fed soy formula would be expected to have high serum levels starting immediately after birth and persisting until they were weaned, at which time levels would drop and likely remain low thereafter.

ESTROGEN RECEPTOR STATUS

Epidemiological studies have found that several hormone-related lifestyle factors, such as nulliparity, earlier age at menarche, higher body mass index (BMI), and use of postmenopausal hormones were related to elevated risk of estrogen receptor-positive, but not estrogen receptor-negative, cancers.[62,63] Because isoflavones have an affinity for estrogen receptors, the protective effect of soy intake may be pronounced for estrogen receptor-positive tumors. To date, 6 studies have assessed the association of soy or isoflavone with breast cancer risk, with regard to the estrogen receptor status of tumors.[34,64–68] Some of these studies, including 1 conducted in Japan, observed an inverse association of soy intake with the risk of estrogen-positive tumors,[64,65,67] but not with the risk of other tumor types.[34,66,68] The incidence rate of estrogen receptor-positive tumors is increasing in the United States.[69] In addition, it has been reported that the prevalence of estrogen receptor-positive expression in normal breast tissue from Japanese women was lower than that in white women.[70] This may simply be a result of the lower incidence of breast cancer among Japanese women. The determinants of estrogen receptor status in normal breast tissue and tumors are not known.

HORMONAL PROFILE

It has been suggested that isoflavones have stimulatory effects in low-estrogen environments, and that in high-estrogen environments, they block the effects of estrogen.[71] Evidence from case-control studies has supported a protective role for soy in premenopausal women versus postmenopausal women.[10] The effects of soy and isoflavone on the level of circulating estrogen have been examined in many intervention studies. A recent meta-analysis of intervention studies revealed that although soy and isoflavone consumption did not affect estradiol or estrone, it did reduce FSH and LH in premenopausal women.[72] Moreover, in postmenopausal women, soy and isoflavone were associated with a small but nonsignificant increase in total estradiol. These observations lend tentative support to the above hypothesis. However, in a cohort study that observed a significant inverse association between soy isoflavone intake and breast cancer risk among Japanese women, the association was somewhat stronger in postmenopausal women than in premenopausal women.[12] A similar tendency was noted in another recent cohort study of Asian women.[68] In addition, some studies have observed lower endogenous estradiol levels in low-risk populations than in high-risk populations.[73,74] Although postmenopausal estrogen levels should not be compared when measured by different laboratories, estrogen levels appear to be low among postmenopausal Japanese women (ie, estradiol <6 pg/mL and estrone <10 pg/mL).[75–79] Such a low-estrogen environment, which likely reflects low body fat mass, may favor the protective effects of soy on breast cancer in Japanese women. Some studies of soy and breast cancer risk included analyses stratified by BMI but not by estrogen level. The results were not consistent: one observed a somewhat stronger association in women with high BMI,[55] but others noted no significant differences.[68,80]

CONCLUSIONS

The causes of breast cancer remain unclear. Established risk factors include age, race/ethnicity, reproductive factors, and obesity. Unfortunately, there are few modifiable factors. Dietary factors have been implicated in the etiology of breast cancer and soy/isoflavone has been a candidate for dietary intervention. However, existing evidence from epidemiological studies is not conclusive and there have been few prospective studies of the issue. Estimation of soy and isoflavone intake, like other dietary components, is subject to measurement error. In addition, the existence of hidden sources of soy makes it difficult to estimate total isoflavone intake accurately, especially among "nonconsumers" of soy-based foods.

There is a need for more, methodologically sound, prospective studies—with extensive exposure measurement. To interpret the data, we need to consider the factors described above, namely, isoflavone dose, forms and sources of isoflavone, timing of isoflavone exposure, and the equol-producer status, estrogen-receptor status, and hormonal profile of individuals, as these are all factors that potentially modulate the association between soy intake and breast cancer risk. Other dietary, environmental, and genetic factors may also modify the association. Future studies need to address these questions by including samples large enough to detect the factors that are capable of modifying the associations between soy and breast cancer risk.

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Association of dietary fat, vegetables and antioxidant micronutrients with skin ageing in Japanese women

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Daily diet may have implications for skin ageing. However, data on the relationship between diet and the parameters of skin conditions are scarce. The present study aimed to examine the associations of biophysical properties of the skin of women with intakes of fats and antioxidant micronutrients as well as food groups as sources of these nutrients. In a cross-sectional study, we measured the hydration, surface lipids and elasticity of the skin of 716 Japanese women using non-invasive techniques. The extent of facial wrinkles in the crow's-foot area was determined by observation using the Daniell scale. Each subject's usual diet was determined with the use of a validated FFQ. After controlling for covariates including age, smoking status, BMI and lifetime sun exposure, the results showed that higher intakes of total fat, saturated fat and monounsaturated fat were significantly associated with increased skin elasticity. A higher intake of green and yellow vegetables was significantly associated with a decreased Daniell wrinkling score. Intake of saturated fat was significantly inversely associated with the Daniell wrinkling score after additional adjustment for green and yellow vegetable intake. Further studies with more accurate measurement methods are needed to investigate the role of daily diet in skin ageing.

Dietary fats: Skin elasticity: Facial wrinkling: Vegetables

Interest in the impact of ageing on the function and appearance of the skin has been increasing[1]. Aged skin has several typical characteristics, including fine wrinkles, dryness, sallowness and loss of elasticity[2]. These characteristics are thought to result from a complex process controlled by both environmental and genetic factors[3]. Among environmental factors, sun exposure and cigarette smoking have long been investigated as risk factors for premature skin ageing[4–6]. Large amounts of micronutrients such as those with antioxidant capacity are present in the skin and are suggested to contribute to the maintenance of skin health[7]. *n*-3 PUFA, especially EPA and DHA, are known to have anti-inflammatory effects and thus are thought to have a protective effect against inflammatory skin disorders[8]. The effects of the antioxidant vitamins and carotenoids or fish oil on conditions of the skin have been examined in some intervention studies[9]. However, these studies are limited by the use of supplements. Little is known about the effects of the long-term consumption of vitamins, carotenoids, or fatty acids at nutritional amounts in the daily diet.

To our knowledge, only three studies have examined associations between daily diet and skin conditions[10–12]. Purba *et al.*[10] assessed actinic skin wrinkling of the back of the hand based on the grading of cutaneous microtopographs of 453 men and women living in Europe and Australia. Using a non-invasive technique, Boelsma *et al.*[11] measured the hydration and surface pH of the skin of the right arm and the sebum content of the forehead of 302 Dutch men and women. Cosgrove *et al.*[12] assessed the appearance of wrinkles, senile dryness and skin atrophy, which were classified as present or absent based on observations by dermatologists, among 4025 American women. These studies revealed that certain types of fats or antioxidant micronutrients were associated with some measured skin properties. However, considering that the sites of the skin measured, the parameters of skin conditions, and the skin assessment methods varied among these studies, cumulated data are still scarce and far from conclusive. In the present study of Japanese women, we examined cross-sectional relationships between dietary intakes of fats and antioxidant micronutrients as well as food groups as sources of these nutrients and biophysical properties of the skin, including hydration, sebum content, elasticity and wrinkle appearance.

Materials and methods

Study population

The present study was part of a larger study designed to assess the relationships among lifestyle, environmental factors and

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women's health[13]. Study subjects were participants in a medical health check-up programme provided by a general hospital in Gifu, Japan, between October 2003 and March 2006. A total of 2073 individuals, including return visitors to the programme during the study period, were invited to join the study, and 1545 agreed to participate (response proportion: 74·5 %). When the response proportion was calculated for only new visitors to the programme during the study period, it was 83·2 % (1103 out of 1325 individuals). The details are described elsewhere[13]. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethical board of the Gifu University Graduate School of Medicine. Written informed consent was obtained from all subjects.

Dietary measurement

The participants responded to a self-administered questionnaire that included questions on demographic characteristics, smoking and drinking habits, diet, exercise, sun exposure, and medical and reproductive histories. Intakes of foods and nutrients were estimated based on a validated 169-item semi-quantitative FFQ using the Japanese Standard Tables of Food Composition, 4th and 5th editions, and fatty acid composition data published by Sasaki *et al.* [14]. Intake of long-chain *n*-3 PUFA was calculated as the sum of EPA and DHA. Detailed information on the questionnaire, including its validity and reproducibility examined in other samples, has been described elsewhere[15]. The Spearman correlation coefficients between the questionnaire and twelve daily diet records kept over a 1-year period for major and micronutrients ranged from 0·29 for carotene (α- and β-carotenes plus cryptoxanthin; expressed as β-carotene equivalents) to 0·73 for Ca. Those for intakes of total fat, saturated fats, monounsaturated fat, polyunsaturated fat, and green and yellow vegetables were greater than 0·50. Our questionnaire was designed to measure an individual's relative intake of foods and nutrients rather than absolute values. The means estimated from the FFQ were generally higher than those estimated from twelve daily diet records. Although we presented the mean values of dietary intake, some of them may have been overestimated by our questionnaire.

Assessment of other exposure variables

Data on lifetime sun exposure were collected through face-to-face interviews. Subjects identified periods in their lives during which they had had stable patterns of outdoor activity. For each of these periods, the subjects were asked about the amount of time they spent outdoors per d during a typical week. Weekday and weekend exposures were recorded separately. We estimated the total time spent outdoors from age 12 years until the day of the interview. Current use of sunscreens during summer was also recorded. Exercise was assessed by asking the average time spent (h per week) performing various kinds of activities during the past 1 year. The details, including validity, are described elsewhere[16].

Assessment of skin ageing

Skin properties were measured by two investigators. The subjects rested for about 15 min in a room for skin measurements. The cosmetics on the selected sites were removed by the investigators. All the measurements were performed in triplicate.

Skin hydration was measured with a corneometer (CM825; Courage and Khazaka, Electronic GmbH, Cologne, Germany). The probe was applied to the skin of the inner side of the right upper arm for 1 s at a pressure of 3·5 N/cm^2. The degree of epidermal humidity is indicated in system-specific units.

Skin surface lipids composed of sebum and corneal lipids were measured with a Sebumeter (SM810; Courage and Khazaka, Electronic GmbH). This device, which works on the principle of photometry, has a special plastic strip that becomes transparent when it absorbs fat. The measuring head equipped with this strip was pressed on the skin of the forehead with a constant pressure of 10 N/64 mm^2 for 30 s. The value displayed corresponded to the amount of sebum on the skin surface in μg/cm^2.

Skin elasticity of the inner side of the right upper arm was measured with a cutometer (SEM575; Courage and Khazaka, Electronic GmbH). The measurements were performed with a negative pressure of 350 mbar, a suction time of 2 s and a relaxation phase of 2 s. The assessment parameters consisted of the Ur/Uf coefficient, which represents the ability of the skin to regain its initial position after deformation.

The extent of facial wrinkles in the crow's-foot area was determined by the Daniell scale[4] ranging from grade I (essentially no wrinkling) to grade VI (profound wrinkling extending over most of the face).

Data analyses

A total of 1086 women participated in the skin measurements. Women who had cancer (*n* 24) or collagen diseases (*n* 11) such as rheumatism and systemic lupus erythematosus were excluded from the present analyses. Additionally, 319 women who had not responded to the dietary questionnaire (*n* 259) or those with incomplete or unreliable responses to the dietary questionnaire (criteria shown elsewhere[17]; *n* 60) and sixteen women who did not complete the interview of lifetime sun exposure were excluded from the present analyses. Thus, 716 women aged 20–74 years comprised the study population. Skin elasticity data were unavailable for 124 women as the device was out of order during their check-ups. Women who did not respond to the dietary questionnaire were more likely to be older than those who did. After controlling for age, the means of skin property variables of the non-responders were similar to those who responded to the dietary frequency questionnaire except for a lower mean value for skin surface lipids.

For statistical analysis, dietary intakes were adjusted for total energy after log-transformation by using the residual method proposed by Willett[18]. Quintiles of the dietary variables were derived based on their distribution in the total population. The means of skin properties for each quintile were provided using analysis of covariate models. A linear trend was assessed using continuous values. Since skin hydration was correlated with room temperature and humidity

Table 1. Characteristics of study subjects and their correlations with skin parameters (*n* 716)
(Mean values and standard deviations, percentages and correlations)

	Characteristics		Correlations†			
	Mean	SD	Hydration	Surface lipids	Elasticity	Facial wrinkles
Age (years)	43·3	8·2	0·04	−0·20**	−0·32**	0·70**
BMI (kg/m^2)	21·3	2·9	0·12	0·02	0·13**	0·03
Education (years)	13·7	2·0	−0·14	0·005	−0·02	0·02
Exercise (METs h/week)	26·2	33·0	−0·0003	−0·03	−0·06	−0·07
Sun exposure (cumulative h)	17430	10264	0·01	0·02	−0·01	0·17**
Alcohol intake (ml/d)	6·6	16·0	0·03	0·05	−0·03	0·05
Not married (%)	18·7		−0·04	0·001	−0·07	−0·03
Postmenopausal (%)	19·1		−0·03	−0·05	−0·03	0·04
Current smokers (%)	6·0		−0·01	0·07	−0·03	0·10**
Ex-smokers (%)	3·9		0·02	−0·01	0·005	0·03
Current HRT use (%)	3·4		0·03	0·05	0·01	−0·05
Current OC use (%)	0·8		0·01	0·002	0·005	−0·03

METs, metabolic equivalents, HRT, hormone replacement therapy; OC, oral contraceptives.
** $P<0·01$.
† Spearman correlation coefficients were adjusted for age, except for age. Dummy variables were given for categorical variables.

(Spearman's r were −0·19 and 0·62, respectively), the results under restricted ranges (temperature, 20–25°C; humidity, 40–60 %; n 209) are shown. Room temperature and humidity were dealt with as covariates because they were strongly correlated with skin hydration (r 0·09 for temperature and r 0·50 for humidity). Although the correlation coefficients of room temperature and humidity with other skin variables were less than 0·08, they were also included as covariates. The known or suspected risk factors for skin conditions, such as age, BMI, smoking status and sunlight exposure[19], were included in the models as covariates in addition to room temperature and humidity. The indicator for raters was also included as a covariate. All the statistical analyses were performed using SAS programs (SAS Institute Inc., Cary, NC, USA). Significance was defined as two-sided $P<0·05$.

Results

Table 1 shows the characteristics of the study subjects and their correlations with skin parameters. Age, BMI, smoking status and sunlight exposure were significantly correlated with one or more parameters. Table 2 shows the intercorrelations among the skin parameters.

Table 3 shows the means for each skin parameter according to the quintile of the selected nutrient or food intake after controlling for the covariates. Surface lipids were significantly positively associated with fresh and processed meats. Skin elasticity was significantly positively associated with total fat, saturated fat and monounsaturated fat, although the differences in the mean elasticity between the highest and the lowest quintiles of these types of fats were less than 2 %. The Daniell wrinkling score was significantly inversely associated with green and yellow vegetable intake. Saturated fat was marginally significantly associated with Daniell's wrinkling score, but this association obtained significance after additional adjustment for green and yellow vegetable intake ($P=0·049$). The association between the Daniell wrinkling score and green and yellow vegetable intake remained statistically significant after additional adjustment for saturated fat intake ($P=0·04$). Vitamins C and E and Zn were unrelated to any parameter of the skin measurements.

Discussion

Skin elasticity on the forearm has been reported to decrease with chronological age[20,21]. In the present study, skin elasticity was significantly inversely correlated with facial wrinkle score, suggesting the importance of this parameter in skin ageing. We observed that higher intakes of total fat, saturated fat and monounsaturated fat were moderately but significantly associated with increased elasticity. A higher intake of long-chain n-3 fatty acids was marginally significantly associated with increased elasticity. The associations with elasticity did not differ greatly by type of fat, although polyunsaturated fat was not significantly associated with skin elasticity. Fats provide building blocks for many components of epidermal and dermal tissues, and they are sources of energy in cell proliferation, maturation and homeostasis[22]. Fats are sensitive to the oxidation process[22]. However, maintenance of collagen and elastic fibres may require adequate amount of fat. Higher saturated fat intake was also significantly associated with a decreased facial wrinkling, suggesting a favourable effect of fat. None of the previous cross-sectional studies on diet and skin conditions[10–12] included the measurement of skin elasticity but addressed the association of fat intake with other parameters of skin conditions. Purba *et al.*[10] found that higher intakes of total fat and monounsaturated fat were significantly associated with decreased wrinkling on

Table 2. Intercorrelations among skin parameters
(Spearman correlation coefficients)

	Hydration	Surface lipids	Elasticity	Facial wrinkles
Hydration	1·00	0·02	0·03	0·12
Surface lipids	–	1·00	0·13**	−0·11**
Elasticity	–	–	1·00	−0·30**
Facial wrinkles	–	–	–	1·00

** $P<0·01$.

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Table 3. Adjusted* means of skin parameters according to quintiles (Q) of selected dietary variables

	Median†	Hydration (arbitrary units)	Surface lipids (μg/cm²)	Elasticity (%)	Facial wrinkles (Daniell score)
Total fat (g/d)					
Q1	50·8	41·4	68·6	81·8	2·48
Q2	57·3	41·6	69·8	82·4	2·48
Q3	62·4	40·2	75·9	83·1	2·57
Q4	67·2	40·2	73·3	82·8	2·41
Q5	73·8	40·0	72·3	83·6	2·38
P for trend		0·28	0·26	0·007	0·17
Saturated fat (g/d)					
Q1	13·6	41·3	68·2	82·0	2·51
Q2	16·2	41·5	69·5	82·8	2·54
Q3	18·2	39·3	72·8	82·7	2·43
Q4	19·8	41·5	77·0	82·9	2·45
Q5	23·4	40·4	72·3	83·3	2·39
P for trend		0·64	0·12	0·03	0·06
Monounsaturated fat (g/d)					
Q1	16·3	41·3	68·2	81·7	2·54
Q2	19·2	42·0	70·9	82·2	2·46
Q3	21·2	40·5	72·8	83·2	2·44
Q4	23·2	39·4	74·4	83·0	2·52
Q5	26·1	40·0	73·5	83·6	2·36
P for trend		0·18	0·07	0·007	0·34
Polyunsaturated fat (g/d)					
Q1	12·1	42·8	74·5	82·5	2·43
Q2	14·0	39·8	72·0	82·3	2·51
Q3	15·3	40·3	70·1	82·6	2·44
Q4	16·6	40·7	70·9	83·0	2·45
Q5	18·9	39·8	72·3	83·2	2·48
P for trend		0·27	0·72	0·19	0·11
Long-chain *n*-3 fatty acids (mg/d)					
Q1	366	42·0	70·1	81·9	2·46
Q2	508	41·4	71·2	82·6	2·49
Q3	630	40·1	79·0	83·5	2·44
Q4	817	39·2	70·7	82·7	2·52
Q5	1121	40·7	68·7	83·0	2·41
P for trend		0·21	0·87	0·09	0·95
Carotene (mg/d)					
Q1	2973	41·6	69·7	82·6	2·54
Q2	4014	40·0	77·3	83·4	2·57
Q3	4848	39·7	67·4	82·5	2·45
Q4	6188	40·6	73·9	82·6	2·39
Q5	8820	41·2	71·5	82·6	2·36
P for trend		0·69	0·53	0·56	0·08
Vitamin C (mg/d)					
Q1	90·9	41·4	73·4	82·9	2·48
Q2	115·6	38·7	63·7	82·5	2·43
Q3	141·8	41·8	74·8	82·3	2·51
Q4	170·2	40·3	72·8	82·7	2·35
Q5	235·3	41·0	75·1	83·1	2·55
P for trend		0·95	0·47	0·76	0·78
Vitamin E (mg/d)					
Q1	8·4	40·6	74·8	82·3	2·51
Q2	9·6	41·2	70·9	82·8	2·54
Q3	10·4	40·3	73·9	82·9	2·52
Q4	11·5	40·0	70·4	82·8	2·30
Q5	13·2	41·3	69·8	82·9	2·45
P for trend		0·88	0·54	0·49	0·27
Zn (mg/d)					
Q1	9·4	41·2	69·1	82·5	2·56
Q2	10·2	39·0	72·1	82·9	2·47
Q3	10·9	40·9	71·5	82·5	2·41
Q4	11·5	41·5	72·0	82·5	2·47
Q5	12·6	40·4	75·2	83·3	2·40
P for trend		0·53	0·36	0·18	0·36
Fresh and processed meats (g/d)					
Q1	37·9	40·9	64·0	82·6	2·38
Q2	58·3	42·1	72·1	82·6	2·46
Q3	72·4	41·9	71·2	82·4	2·50
Q4	90·6	38·3	73·2	82·8	2·59
Q4	123·0	39·5	79·5	83·3	2·39
P for trend		0·25	0·01	0·37	0·65

Table 3. *Continued*

	Median†	Hydration (arbitrary units)	Surface lipids (μg/cm²)	Elasticity (%)	Facial wrinkles (Daniell score)
Fish and shell fish (g/d)					
Q1	40·5	41·9	77·6	82·9	2·35
Q2	58·0	38·8	67·0	82·0	2·46
Q3	71·4	40·9	74·2	83·2	2·56
Q4	86·0	40·8	70·2	82·8	2·50
Q5	114·4	41·0	70·6	82·7	2·46
P for trend		0·29	0·37	0·56	0·42
Green and yellow vegetables (g/d)					
Q1	62·9	40·3	71·6	82·5	2·51
Q2	91·7	41·4	75·9	83·6	2·58
Q3	120·1	42·1	66·7	82·4	2·46
Q4	156·9	38·2	70·1	82·9	2·40
Q5	250·2	41·6	75·5	82·3	2·37
P for trend		0·82	0·36	0·91	0·04
Other vegetables (g/d)					
Q1	121·4	41·1	72·1	82·8	2·48
Q2	179·5	39·1	79·8	83·2	2·49
Q3	206·3	41·2	70·6	82·6	2·41
Q4	247·1	40·7	66·8	82·4	2·41
Q5	335·6	41·4	70·5	82·7	2·52
P for trend		0·45	0·57	0·32	0·73
Fruits (g/d)					
Q1	34·6	40·2	77·6	82·5	2·56
Q2	61·3	39·8	66·3	83·0	2·43
Q3	89·4	40·8	68·9	82·1	2·42
Q4	140·3	41·6	71·9	82·8	2·47
Q5	229·0	40·9	75·0	83·3	2·43
P for trend		0·55	0·69	0·12	0·24

* Adjusted for total energy, age, BMI, smoking status, cumulative sun exposure, rater, and room temperature and humidity.
† Adjusted for total energy.

the back of the hand; this result does not contradict the present results. However, a higher intake of butter or margarine was associated with increased wrinkling. Boelsma *et al.* [11] observed that higher intakes of total fat, saturated fat and monounsaturated fat were significantly associated with decreased skin hydration of the skin on the right arm. In the study reported by Cosgrave *et al.* [12], a higher intake of fat was associated with wrinkled appearance and senile dryness, but a higher intake of linoleic acid was associated with decreased senile dryness. The results of these studies suggested an unfavourable role of certain types of fat in skin health. Fat consumption in the present study subjects, like that in other Japanese populations, was low as compared with those among Western populations, which may partially explain the discrepancies in the results.

Skin elasticity has been reported to be improved by hormone replacement therapy[23,24]. Higher fat intake has been associated with increased endogenous oestrogen concentrations of women in some studies[25,26]. The observed association of fat intake with skin elasticity may be explained by oestrogen profile. We expected that alcohol and phyto-oestrogens such as soya isoflavones might mimic the effects of oestrogen on the skin. However, neither alcohol nor dietary soya was associated with skin elasticity or the other parameters (data not shown).

Dietary supplementation with antioxidant vitamins or carotenoids has shown photoprotective effects on the skin in some studies[27–29]. In the present study, a higher intake of green and yellow vegetables was associated with decreased facial wrinkling. Carotene, which is abundant in green and yellow vegetables, was marginally inversely associated with facial wrinkling. However, we cannot deny a possibility that the observed inverse association of green and yellow vegetable intake with facial wrinkling may be due to certain nutrients other than carotene. Purba *et al.* [10] also observed that a higher intake of vegetables was associated with less actinic damage of the back of the hand.

The FFQ, like all methods of dietary assessment, is subject to measurement error. In addition, the measurements of skin properties are likely to be affected by environmental conditions, such as the temperature and humidity of the room for the measurements. A room humidity of between 40 and 60 % is generally recommended for measurements of skin hydration[30]. However, we noticed that the room humidity affected the skin hydration, even in such a restricted range. Nonetheless, the findings were not altered in subanalyses with narrow ranges for room temperature and humidity. It is unlikely that the measurement errors in the skin parameters are dependent on diet. Thus, the observed associations were modest but likely to be underestimated. The homogeneity of diet among the study subjects might have precluded us detecting a significant association.

Because of the cross-sectional nature of the data, no causal inferences can be drawn regarding any of the associations observed. The relatively narrow age range and the inclusion of women only are also limitations. Although fats and green and yellow vegetables were associated with some skin parameters of Japanese women, these modest associations may be by chance due to multiple testing. Given the small number of epidemiological studies on diet and skin health

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and the potential for these studies to promote a healthy diet, further studies with more accurate measurement methods are needed.

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Consumption of coffee, green tea, oolong tea, black tea, chocolate snacks and the caffeine content in relation to risk of diabetes in Japanese men and women

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Although the inverse association between coffee consumption and risk of diabetes has been reported numerous times, the role of caffeine intake in this association has remained unclear. We evaluated the consumption of coffee and other beverages and food containing caffeine in relation to the incidence of diabetes. The study participants were 5897 men and 7643 women in a community-based cohort in Takayama, Japan. Consumption of coffee, green tea, oolong tea, black tea and chocolate snacks were measured with a semi-quantitative FFQ in 1992. At the follow-up survey in 2002, the development of diabetes and the time of diagnosis were reported. To assess the association, age, smoking status, BMI, physical activity, education in years, alcohol consumption, total energy intake, fat intake and women's menopausal status were adjusted. Among men who consumed one cup per month to six cups per week and among those who consumed one cup per d or more, the associated hazard ratios were 0·69 (95 % CI 0·50, 0·97) and 0·69 (95 % CI 0·49, 0·98) compared with those who drank little to no coffee, with a P value for trend of 0·32. The hazard ratios for women with the same coffee consumption patterns were 1·08 (95 % CI 0·74, 1·60) and 0·70 (95 % CI 0·44, 1·12), with a P value for trend of 0·03. The association between estimated total caffeine intake and risk of diabetes was insignificant both among men and among women. The results imply that coffee consumption decreased the risk of developing diabetes. The protective effect may exist aside from the influence of caffeine intake.

Coffee: Diabetes mellitus: Caffeine: Japanese

The protective effect of heavy consumption of coffee on the development of diabetes has been reported in many epidemiological studies, substantially from European countries and the USA, where coffee is widely consumed[1–8]. Because caffeine is one of the biologically active components in coffee, its role in the association with diabetes has also been investigated as described below. Several clinical studies have shown that oral administration of caffeine increases thermogenesis and metabolism[9–11]. These results may support the protective effect of caffeine intake over the risk of diabetes through reducing the risk of obesity, although the studies have expressed a rather short-term effect. On the other hand, several other studies have shown that caffeine intake causes the reduction of glucose disposal and increases insulin resistance[12–14]. To assess the long-term effects of caffeine intake, observational studies have been conducted to examine the relationship between caffeine intake and the development of diabetes. The results of some of the studies indicated a lowered risk of diabetes with increased caffeine intake[15–19]. On the contrary, several studies showed that decaffeinated coffee also decreased the risk of diabetes[18–20].

Caffeine is also contained in other dietary items such as tea and chocolate. In contrast to people in several European countries and the USA, tea is commonly consumed in Japan, and, hence, it should also be considered as its source to evaluate caffeine intake among Japanese subjects. Three previous studies among Japanese people evaluated green tea consumption with the risk of diabetes, but the results were inconsistent[15,16,21]. Studies in the USA implied a protective effect of tea consumption on diabetes risk, although the upper 95 % CI was at the null value; the hazard ratio was 0·77 (95 % CI 0·59, 1·00) for a two cups per d increment in intake in one study[17], and it was 0·88 (95 % CI 0·64, 1·23) for four or more *v.* no cups per d in the other study[19]. Another study in the USA failed to show the association between tea consumption and risk of diabetes[18]. Chocolate snacks are relatively common in Japan, although the reported per capita consumption has been found to be lower than that in most Western countries: 23 % of the consumption in the UK, and 37 % of the consumption in the USA[22].

We assessed the association between coffee consumption and risk of developing diabetes in a prospective cohort study among men and women in a general Japanese population. We further evaluated the consumption of beverages and foods containing caffeine. Total caffeine intake was estimated and discussed in relation to the risk of diabetes.

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Materials and methods

Subjects in the present study were from a community-based cohort study conducted in Takayama City (Gifu, Japan). The rationale and design of the Takayama study have been described in detail elsewhere[23–25]. In 1992, 31 152 individuals aged 35 years and over completed a baseline self-administered questionnaire which included a semi-quantitative FFQ for 169 food items consumed in the previous year, and other questions asking about physical and demographic characteristics such as age, height, weight, marital status and length of education. Women's health issues including menopausal status and use of hormone replacement therapy were also asked. The questionnaire also asked about smoking status, previous diagnosis of diabetes and other medical and reproductive histories. To assess the amount of regular physical activity, the average time (in hours) spent for listed physical activities was sought, and the metabolic equivalents were estimated. The list contained vigorous sports (such as jogging, bicycling on hills, tennis, racquet ball, swimming laps, or aerobics), vigorous work requiring muscle strength and endurance (such as moving heavy furniture, loading or unloading trucks, shovelling, or other equivalent manual labour) and moderate sports or work (such as housework, brisk walking, golfing, bowling, bicycling on level ground, or gardening). Further details and the validity information of the physical activity questionnaire have been previously reported[26,27]. The participation rate for the questionnaire administered at baseline was 85·3 %. In the cohort, participants who were younger than 70 years at baseline (*n* 26 546) were followed for the present study. Among them, 1120 participants died and 1058 participants moved out of Takayama between September 1992 and March 2000, as confirmed by the residential registry. For the remainder of the follow-up until July 2002, we did not have access to the residential registry, but we identified an additional 404 deaths using the obituaries issued by Takayama city. After excluding the deaths and relocations, we sent 23 964 participants a follow-up questionnaire in 2002. In response to sending out the questionnaire, we learned that an additional 1460 participants had moved out of Takayama, eighteen had died and fifty-one were physically unable to complete the questionnaire. Of the remaining subjects, 14 975 completed the questionnaire, which yielded the response rate of 66·7 %. Compared with the 14 975 subjects, the 11 571 subjects without follow-up data were slightly younger (aged 50·3 *v.* 52·3 years among men and aged 50·8 *v.* 52·0 years among women), less likely to be educated 12 years or longer (43·9 *v.* 46·9 % among men and 35·5 *v.* 39·4 % among women), more likely to have high caffeine intake (139 *v.* 132 mg among men and 144 *v.* 138 mg among women), but were similar in terms of BMI.

For the present analysis, participants who reported a diagnosis of diabetes (*n* 541), cancer (*n* 274), or either myocardial infarction, angina or stroke (*n* 535) at baseline were excluded. We further excluded participants who were newly identified having diabetes at baseline from the follow-up questionnaire (*n* 85). After these exclusions, 5897 men and 7643 women were included in the present analysis.

The information on baseline consumption of coffee and other beverages and foods among the participants was derived from the FFQ administered at baseline. The validity and reliability of the questionnaire and other detailed information have been described previously[24]. In the present study, we evaluated the following drinks: coffee, decaffeinated coffee, green tea, oolong tea and black tea. We also examined the consumption of chocolate snacks, since chocolate is also a source of caffeine[28,29]. Chocolate truffles and solid chocolate bars are common chocolate snacks in Japan. Since there were separate questions for cookies/biscuits and cake, chocolate cookies, chocolate-covered cookies and chocolate cake were not likely to be classified as chocolate snacks by many participants. The content of caffeine from coffee and tea was estimated by using data from the Standard Tables of Food Composition in Japan, 5th edition, published by the Science and Technology Agency of Japan. In the questionnaire, one serving was defined as 150 g for coffee and decaffeinated coffee, 100 g was defined as one serving for green tea and black tea, and 250 g was defined as one serving for oolong tea. The estimated content of caffeine per serving was 90 mg for coffee, 20 mg for green tea, 30 mg for black tea and 50 mg for oolong tea. The consumption of decaffeinated coffee was asked separately from the consumption of coffee, and, hence, estimated consumption of coffee and that of decaffeinated coffee were mutually exclusive. The caffeine content in chocolate snacks was defined as 12·5 mg per 100 g according to a literature review[28]. We estimated the intake of caffeine from each beverage and chocolate snack by totalling a weight proportional to the frequency of consumption in the questionnaire, and multiplying that total by the above caffeine content. We also estimated the intake of other nutrients based on the FFQ by referring to the same standard table. The intake of each nutrient was adjusted for total energy after log-transformation by using the residual method proposed by Willett[30].

The participants who developed diabetes between the time of the baseline study and at the time of follow-up were identified in the questionnaire. All participants were asked if they had ever been diagnosed with diabetes, and, if so, how old they were at the time of the diagnosis. Using the information on their age at baseline and age at diagnosis, the time from baseline to diagnosis was estimated. Because thirty-one men and ten women who developed diabetes during the follow-up period did not provide the information regarding the time of diagnosis, we assigned median values of length to the diagnosis among participants for men and women separately.

Statistical analysis

Participants were placed into categories based on the frequency of consumption of coffee, tea and chocolate snacks, roughly based on the distribution of consumption of each item in the current population. Three categories for frequency of coffee and oolong tea consumption were created: never or almost never, once per month to six times per week, and once per d or more. Four categories for frequency of green tea consumption were created: never or almost never, once per month to six times per week, once per d, and twice per d or more. For the consumption of decaffeinated coffee and black tea, two categories were created: never or almost never and once per month or more. The amount of chocolate snacks consumed was multiplied by the frequency of consumption, and put into three categories: never or almost never, one piece

per month to two or three pieces per month, and one piece per week or more. Caffeine intake was analysed in tertile groups. Cox proportional hazards models were used to assess the contributions of coffee, tea, chocolate consumption, and caffeine intake respectively, to the subsequent risk of developing diabetes. The age-adjusted model and multivariate model adjusted for potential confounders, age, smoking status, BMI, physical activity, education in years, alcohol consumption, total energy intake, fat intake and women's menopausal status were examined for each beverage and chocolate snacks respectively. To test for linear trends across categories, we modelled the median of each category of coffee, tea and chocolate consumption, and caffeine intake as a continuous variable.

All statistical analyses were performed by using SAS statistical software (version 9.1; SAS Institute, Inc., Cary, NC, USA). Statistical significance was considered to be $P<0.05$. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee at Gifu University Graduate School of Medicine. Written informed consent was obtained from all subjects.

Results

Of the 5897 men and 7643 women participating in the study, 278 men and 175 women reported the development of diabetes during the follow-up. Baseline characteristics of the study participants across sex-specific categories of coffee consumption are presented in Table 1. Higher coffee consumption was associated with younger age, 12 years or more of education, and cigarette smoking. Total energy intake and carbohydrate intake were higher with an increased level of coffee consumption. Consumption of soda was also higher with the increased level. Correlation coefficient analysis between caffeine intake and BMI showed no clear correlation both among men and among women (data not shown).

With the multivariate model, hazard ratios among men showed that participants who consumed coffee once per month to six times per week and who consumed coffee daily had a significantly decreased risk of diabetes compared with those who never or almost never consumed coffee, with no significance in analysis of trend (Table 2). Among women, although hazard ratios did not show a significant association, an analysis for linear trend after multivariate adjustment showed a statistically significant decrease in the development of diabetes (Table 2).

Consumption of decaffeinated coffee tended to be associated with a decreasing risk of diabetes among women, but the association was not statistically significant (Table 2). Green tea consumption and black tea consumption were not significantly associated with a risk of diabetes either. Consumption of oolong tea was insignificantly positively associated with a risk of diabetes among men, and the trend analysis showed the risk increased significantly with higher consumption among women (Table 2). Although the association with consumption of oolong tea was attenuated after multivariate adjustment among men, it remained significant after the adjustment among women. We observed a weak but significant inverse association between the consumption of chocolate snacks and the risk of diabetes among men in the trend analysis. The lowered risk was also observed among women, although not all the decreases in hazard ratios were statistically significant, and no significance was observed in the trend analysis (Table 2).

The association between coffee consumption and risk of diabetes remained in an analysis which included the categorical variables of coffee consumption and caffeine intake simultaneously in the multivariate model. The hazard ratios for diabetes according to coffee consumption categories of never or almost never, once per month to six times per week, and once per d or more were 1·00, 0·70 (95 % CI 0·50, 0·99) and 0·66 (95 % CI 0·43, 1·03), and the P value for trend was 0·33 among men. The corresponding hazard ratios for women were 1·00, 1·00 (95 % CI 0·67, 1·49) and 0·60 (95 % CI 0·36, 1·01), with the P value for trend of 0·02. In the same model, no significant association between caffeine intake and risk of diabetes was observed (data not shown).

To minimise a potential effect of subclinical disease, we conducted additional analyses by excluding thirty-eight men and nineteen women who reported the diagnosis of diabetes during the first 2 years of the follow-up period. The results did not alter our original findings. Because we needed to assign the median length of follow-up to forty-one participants who developed diabetes, we conducted a separate analysis using logistic regression, but these results also did not alter our findings; the multivariate OR for diabetes according to the coffee consumption categories of never or almost never, once per month to six times per week, and once per d or more were 1·00, 0·70 (95 % CI 0·50, 0·98) and 0·69 (95 % CI 0·48, 0·99), and the P value for trend was 0·31 among men. Among women, the same results were 1·00, 1·00 (95 % CI 0·67, 1·48) and 0·60 (95 % CI 0·37, 0·96), with a P value for trend of 0·01.

Discussion

Higher coffee consumption modestly decreased the risk of development of diabetes both among men and women even though consumption of coffee is relatively low in the current Japanese population compared with that in Western countries. In contrast, caffeine intake estimated from coffee, green tea and other caffeinated beverages and chocolate snacks was not associated with risk. The results suggest that a beneficial effect of coffee consumption exists aside from its caffeine content. Studies conducted in the USA showed that a higher caffeine intake significantly lowered the risk of diabetes, but in some of the studies, the association between caffeine intake and risk of diabetes was diminished after further adjustment for coffee consumption[17–19]. In the same studies, it was reported that the consumption of decaffeinated coffee was also inversely associated with the risk of diabetes[17–19]. The consumption of coffee may increase the intake of antioxidants other than caffeine. It has been reported that chlorogenic acid, a polyphenol abundant in coffee, is probably responsible for the substantial part of antioxidants[31–33].

The present study failed to observe any association between decaffeinated coffee and diabetes risk, which may have been caused by a lack of power since a very small number of the participants, less than one-tenth of them, reported consumption of decaffeinated coffee in some frequency. The significant