smoked," "ex-smoker," or "current smoker." Drinking status was classified as "nondrinker" or "current drinker." Educational level, reported as age when education finished, was categorized as <15, 15 to 17, or ≥18 years of age. Anthropometric data were also classified. Body mass index was determined from measurements of body weight and height and was categorized into 3 strata: <18, 18 to <25, or ≥25 kg/m^2. Blood pressure was measured using an automated sphygmomanometer. Hypertension status was classified as the presence of hypertension (systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥90 mm Hg, or taking medication) or absence of hypertension. Blood samples were drawn for measurement of high-density lipoprotein cholesterol levels and blood glucose. Diabetic status was classified as presence of diabetes (fasting blood glucose ≥126 mg/dL, casual blood glucose ≥200 mg/dL, or taking medication) or absence of diabetes.

Ethical Issues

The study design and procedures were reviewed and approved by each municipal government and by the Ethics Committee for Epidemiological Research at Jichi Medical School. Written informed consent to participate in the study was obtained individually from those who responded to the mass screening examination. They were informed that data would be obtained from questionnaires and blood samples. In addition, if ischemic heart disease or a stroke were suspected to have occurred, their health status would be followed up through a review of their hospital medical records.

Follow-up

A mass screening system was used to obtain baseline data and to follow the participants up annually. Death certificates were collected until the end of 2005 from public health centers with the official permission of the Agency of General Affairs and the Ministry of Health, Labour and Welfare. Data on individuals who moved out of the study area were obtained annually from the relevant municipal governments. People who moved out of the communities during the observation period were followed up until the date of the emigration. Causes of death were coded according to the *International Classification of Diseases, 10th Revision* (I00-I99, C00-C97, D00-D48, J00-J99, K00-K93, V01-Y89).

Statistical Analysis

Statistical analyses were performed separately for men and women. First, associations between socioeconomic and behavioral characteristics and soy or soy products intake were evaluated by 1-way analysis of variance or the χ^2 test. Then, Cox proportional hazards regression was used to assess associations between soy or soy products intake and all-cause mortality, cardiovascular mortality, and cancer mortality. Adjustments were made for age, high-density lipoprotein cholesterol, body mass index, drinking status, smoking status, hypertension, diabetes, education level, and menopause (women only). Some individuals with missing data were excluded from the analyses. Hazard ratios (HRs) and 95% confidence intervals (CIs) for mortality were determined. Statistical analyses were performed using IBM SPSS software, version 21.0 J for Windows.

Results

The mean age of the participants at baseline was 54.9 years for men and 55.1 years for women. During a follow-up period over a mean of 11.8 years, 882 deaths were documented. Of these, 213 were from cardiovascular disease (ischemic heart disease: 90; stroke: 108; other: 15), 346 were

from cancer (lung: 76; colon: 31; stomach: 42; other: 197), and 323 were from other causes. Baseline characteristics according to frequency of soy or soy products intake are presented in Tables 1 and 2. Older men and women were more likely to consume soy and soy products. Men and women who consumed soy and soy products most frequently were less likely to ever have been smokers. Men with hypertension were less likely to consume soy and soy products than men without hypertension, but the relationship was nonlinear. Menopausal women consumed soy and soy products more frequently than premenopausal women. Alcohol drinking status and body mass index showed significant nonlinear associations with soy intake.

Crude mortality rates per 1000 person-years and frequency of soy or soy products intake and all-cause mortality in men and women are shown in Tables 3 and 4. Among men, the crude mortality rate was highest in those who reported almost daily intake of soy. Among women, the crude mortality rate was not significantly different between different frequencies of soy intake.

In the primary analysis, only age was adjusted for, and in the subsequent analysis, age, smoking status, drinking status, education level, hypertension, presence of diabetes, high-density lipoprotein cholesterol, body mass index, and menopause (women only) were adjusted for in determining the association between frequency of soy intake and all-cause mortality (Tables 3 and 4). Participants who reported soy intake 1 to 2 times per week were regarded as the reference group. The HR for all-cause mortality in men who reported rarely eating soy was 1.53 (95% CI = 1.13-2.07) and in men who reported almost daily consumption was 1.55 (95% CI = 1.19-2.03) compared with the reference group. The HR for cancer mortality in men who reported rarely eating soy was 1.74 (95% CI = 1.08-2.79) compared with the reference group. No statistically significant association was found between soy intake and cardiovascular mortality in men. In women, no statistically significant association was found between soy intake and all-cause mortality, cardiovascular mortality, or cancer mortality. Soy products intake was not statistically significantly associated with all-cause and cause-specific mortalities in men and women.

The age range of the participants was very wide (19-93 years). Though an adjustment for age has been made in the analyses, residual confounding factors could not be ruled out. Therefore, HRs among those aged 50 years or more were calculated. The results were potentially unchanged (data not shown). In addition, to eliminate the influence of potentially preexisting subclinical diseases, the analyses were repeated after excluding those who died within 3 years of the baseline examination. The results were almost identical (data not shown).

Discussion

In the present study, the relationship between the frequency of soy and soy products intake and mortality was investigated in individuals in a community-based Japanese population. We found that HRs for all-cause mortality were significantly higher in men who reported either infrequent or almost daily soy intake compared with men who reported ingesting soy 1 to 2 times per week. Additional analyses showed that cancer mortality was higher among men who reported rarely eating soy compared with those who reported eating soy 1 to 2 times per week. The similar HRs for all-cause mortality and cancer mortality in those with frequent soy intake suggest that cancer mortality was a major factor in all-cause mortality. There was no statistically significant relationship between soy intake and all-cause, cardiovascular, and cancer mortality in women or with cardiovascular mortality in men. In contrast, associations between soy products and mortality were not statistically significant in men and women. This might have been a result of the variety of different methods used to prepare soy products, such as deep frying or fermentation.

Previous studies have reported a significant association between soy intake and low mortality resulting from various cancers.[9,16] A large-scale cohort study (JACC) reported that a high intake of soy bean curd (tofu) may have preventive effects for ovarian cancer mortality.[17] In contrast,

Table 1. Baseline Characteristics of Study Participants by Frequency of Soy Intake.

	Men, n = 4309						Women, n = 6757					
	Frequency of Soy Intake						Frequency of Soy Intake					
	Rarely	1-2 Times/ Month	1-2 Times/ wk	3-4 Times/ wk	Almost Daily	P[a]	Rarely	1-2 Times/ Month	1-2 Times/ wk	3-4 Times/ wk	Almost Daily	P[a]
Number of individuals	432	1025	1521	878	453		507	1635	2179	1477	959	
Age (year)[b]	53.2 ± 12.3	54.1 ± 11.9	54.1 ± 12.3	56.1 ± 11.4	56.9 ± 11.0	.000[c]	53.3 ± 12.3	54.2 ± 11.7	55.0 ± 11.4	55.9 ± 10.4	56.9 ± 10.4	.000[c]
Smoking status (%)	n = 432	n = 1021	n = 1515	n = 876	n = 450		n = 498	n = 1606	n = 2138	n = 1447	n = 939	
Never smoked	20.8	20.2	21.5	21.9	22.0	.003	84.7	90.5	92.4	93.5	92.8	.000
Ex-smoker	19.9	26.3	28.8	29.2	30.7		4.8	3.3	2.8	2.3	1.6	
Current smoker	59.3	53.5	49.6	48.9	47.3		10.4	6.2	4.9	4.2	5.6	
Drinking status (%)	n = 414	n = 980	n = 1494	n = 853	n = 437		n = 464	n = 1552	n = 2111	n = 1429	n = 923	
Current drinker	74.7	72.5	75.6	75.4	73.7	.429	23.1	26.1	26.8	23.2	22.9	.036
Nondrinker	27.5	24.4	24.6	23.0	26.3		76.9	73.9	73.2	76.8	77.1	
Body mass index (%)	n = 421	n = 1004	n = 1489	n = 860	n = 444		n = 501	n = 1600	n = 2130	n = 1447	n = 946	
<18.0	3.6	1.8	2.5	2.3	3.8	.188	4.4	2.8	3.5	3.5	2.9	.012
18.0 to <25	72.4	77.0	76.1	73.7	73.2		65.5	70.9	73.9	72.0	71.8	
≥25.0	24.0	21.2	21.4	24.0	23.0		30.1	26.2	22.6	24.5	25.4	
Hypertension(%)[d]	n = 428	n = 1012	n = 1502	n = 869	n = 451		n = 506	n = 1622	n = 2155	n = 1462	n = 949	
Yes	60.3	69.7	66.7	61.3	61.0	.000	67.2	68.1	68.7	69.5	66.2	.496
No	39.7	30.3	33.3	38.7	39.0		32.8	31.9	31.3	30.5	33.8	
Diabetes (%)[e]	n = 422	n = 1016	n = 1507	n = 866	n = 450		n = 503	n = 1620	n = 2175	n = 1460	n = 950	
Yes	94.1	94.9	95.8	94.9	92.4	.078	98.2	97.2	97.9	97.3	96.2	.068
No	5.9	5.1	4.2	5.1	7.6		1.8	2.8	2.1	2.7	3.8	
Age (years) when finished education (%)	n = 427	n = 1016	n = 1513	n = 866	n = 444		n = 501	n = 1624	n = 2152	n = 1460	n = 950	
<15	12.9	11.7	11.3	13.3	16.0	.000	25.9	20.9	18.5	18.7	22.8	.001
15-17	56.7	54.4	48.2	50.3	51.1		47.3	46.6	48.0	49.3	47.6	
≥18	30.4	33.8	40.4	36.4	32.9		26.7	32.6	33.4	32.0	29.6	
Menopause	—	—	—	—	—	—	n = 499	n = 1606	n = 2143	n = 1458	n = 949	
Yes	—	—	—	—	—	—	62.3	66.3	68.8	73.5	76.5	.000
No	—	—	—	—	—	—	37.7	33.7	31.2	26.5	23.5	
Lipids	n = 422	n = 1015	n = 1507	n = 867	n = 450		n = 504	n = 1624	n = 2162	n = 1461	n = 950	
High-density lipoprotein cholesterol (mg/dL)[b]	48.2 ± 13.4	48.3 ± 13.8	48.9 ± 12.8	49.5 ± 13.4	49.0 ± 14.3	.120[c]	52.2 ± 12.2	52.7 ± 12.3	52.8 ± 12.4	52.6 ± 12.4	52.8 ± 13.0	.891[c]

[a] χ^2 test.
[b] Mean ± standard deviation.
[c] One-way analysis of variance.
[d] Hypertension: systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥90 mm Hg, or taking medication.
[e] Diabetic: fasting blood glucose ≥126 mg/dL, casual blood glucose ≥200 mg/dL, or taking medication.

Table 2. Baseline Characteristics of Study Participants by Frequency of Soy Products Intake.

	Men, n = 4309				Women, n = 6757			
	Frequency of Soy Intake				Frequency of Soy Intake			
	Less Than 1-2 Times Per Month	1-4 Times Per Week	Almost Every Day	P[a]	Less Than 1-2 Times Per Month	1-4 Times Per Week	Almost Every Day	P[a]
Number of participants	251	2580	1478		244	3523	2990	
Age (year)[b]	51.0 ± 13.8	53.8 ± 12.1	57.4 ± 11.0	.000[c]	55.7 ± 12.3	54.2 ± 11.6	56.2 ± 10.7	.000[c]
Smoking status (%)	n = 251	n = 2571	n = 1472		n = 234	n = 3451	n = 2943	
Never smoked	18.3	20.7%	22.8	.000	85.0	91.6	92.3	.003
Ex-smoker	19.9	27.0%	30.0		4.3	2.7	2.7	
Current smoker	61.8	52.4	47.1		10.7	5.7	5.0	
Drinking status (%)	n = 246	n = 2517	n = 1419		n = 236	n = 3390	n = 2853	
Current drinker	69.9	75.2	76.5	.081	27.5	25.5	24.2	.307
Nondrinker	30.1	24.8	23.5		72.5	74.5	75.8	
Body mass index (%)	n = 248	n = 2518	n = 1452		n = 248	n = 2518	n = 1452	
<18.0	4.4	2.3	2.7	.225	3.4	3.6	2.9	.134
18.0 to <25	72.6	74.9	76.0		73.1	72.5	71.0	
≥25.0	23.0	22.8	21.3		23.5	23.9	26.2	
Hypertension (%)[d]	n = 250	n = 2549	n = 1463		n = 243	n = 3495	n = 2956	
Yes	27.2	33.3	39.2	.000	32.5	30.7	32.9	.174
No	72.8	66.7	60.8		67.5	69.3	67.1	
Diabetes (%)[e]	n = 248	n = 2552	n = 1461		n = 238	n = 3491	n = 2961	
Yes	5.6	4.5	6.1	.960	2.1	2.4	3.0	.289
No	94.4	95.5	93.9		97.9	97.6	97.0	
Age (years) when finished Education (%)	n = 246	n = 2554	n = 1466		n = 239	n = 3485	n = 2963	
<15	10.6	11.7	14.0	.680	24.7	18.5	22.1	.000
15-17	54.5	50.6	52.0		51.5	47.4	48.1	
≥18	35.0	37.6	34.0		23.8	34.1	29.8	
Menopause	—	—	—		n = 238	n = 3465	n = 2952	
Yes	—	—	—		68.1	65.9	74.6	.000
No	—	—	—		31.9	34.1	25.4	
Lipids	n = 422	n = 1015	n = 1507		n = 504	n = 1624	n = 2162	
High-density lipoprotein cholesterol(mg/dL)[b]	45.5 ± 11.9	48.8 ± 13.0	49.5 ± 14.2	.000[c]	52.5 ± 12.7	52.3 ± 12.3	53.1 ± 12.7	.046[c]

[a]χ^2 test.
[b]Mean ± standard deviation.
[c]One-way analysis of variance.
[d]Hypertension: systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥90 mm Hg, or taking medication.
[e]Diabetic: fasting blood glucose ≥126 mg/dL, casual blood glucose ≥200 mg/dL, or taking medication.

the same cohort study reported that consumption of soy food had no protective effects against breast cancer.[18] In addition, the JPHC study reported that frequent miso soup and isoflavone consumption was associated with a reduced risk of breast cancer, but there was no evidence of reduced risk with intake of soy.[19] Moreover, the Whitehall II Study, using the alternative healthy eating index, concluded that soy intake was not associated with cancer mortality.[20] Thus, the issue of whether soy foods promote health and prevent cancer remains controversial. In our findings, intake of soy was not associated with cancer mortality in women. In contrast, men who reported rarely eating soy were at significantly higher risk of cancer mortality than men who reported eating soy 1 to 2 times per week.

As indicated in previous studies, obesity increases the risk of colon cancer and stomach cancer.[21,22] Additionally, both smoking tobacco and consuming alcohol increase the risk of developing lung and other cancers.[23] Therefore, in the present study, we adjusted for body mass index, smoking status, and alcohol consumption; however, the results were generally unchanged.

Table 3. Crude Mortality Rates for 1000 Person-Years, Hazard Ratios, and Frequency of Soy or Soy Products Intake in Men.

	Frequency of Soy Intake				
	Rarely	1-2 Times/Month	1-2 Times/wk	3-4 Times/wk	Almost Daily
Person-years	4932	12 164	18 211	10 362	5153
All causes					
Number of deaths	61	104	164	107	92
Mortality rate	12.4	8.5	9.0	10.3	17.9
HR age (95% CI)	1.54 (1.15-2.07)	1.02 (0.79-1.03)	Reference	1.04 (0.82-1.33)	1.58 (1.23-2.04)
HR all (95% CI)	1.53 (1.13-2.07)	0.94 (0.73-1.22)	Reference	1.04 (0.81-1.33)	1.55 (1.19-2.03)
Cardiovascular					
Number of deaths	11	18	37	22	20
Mortality rate	2.2	1.5	2.0	2.1	3.9
HR age (95% CI)	1.06 (0.55-2.06)	0.73 (0.42-1.25)	Reference	0.88 (0.54-1.45)	1.39 (0.83-2.33)
HR all (95% CI)	1.08 (0.55-2.13)	0.64 (0.35-1.17)	Reference	0.96 (0.57-1.61)	1.39 (0.78-2.41)
Cancer					
Number of deaths	27	42	62	44	32
Mortality rate	5.5	3.5	3.4	4.2	6.2
HR age (95% CI)	1.79 (1.14-2.81)	1.07 (0.72-1.58)	Reference	1.13 (0.77-1.67)	1.47 (0.96-2.26)
HR all (95% CI)	1.74 (1.08-2.79)	0.99 (0.66-1.49)	Reference	1.14 (0.77-1.70)	1.39 (0.88-2.19)
Others					
Number of deaths	23	43	59	39	38
Mortality rate	4.7	3.5	3.2	3.8	7.4
HR age (95% CI)	1.63 (1.01-2.64)	1.17 (0.79-1.74)	Reference	1.06 (0.71-1.59)	1.84 (1.22-2.77)
HR all (95% CI)	1.61 (0.99-2.62)	1.09 (0.72-1.62)	Reference	0.97 (0.64-1.47)	1.82 (1.21-2.77)

	Frequency of Soy Products Intake		
	Less Than 1-2 Times Per Month	1-4 Times Per Week	Almost Every Day
Person-years	2900	30 661	17 260
All causes			
Number of deaths	30	286	212
Mortality rate	10.3	9.3	12.3
HR age (95% CI)	1.31 (0.90-1.90)	Reference	1.07 (0.92-1.33)
HR all (95% CI)	1.27 (0.87-1.85)	Reference	1.11 (0.90-1.28)
Cardiovascular			
Number of deaths	8	62	49
Mortality rate	2.76	2.02	2.84
HR age (95% CI)	1.62 (0.77-3.38)	Reference	1.11 (0.76-1.61)
HR all (95% CI)	1.77 (0.84-3.74)	Reference	1.18 (0.84-1.75)
Cancer			
Number of deaths	8	113	86
Mortality rate	2.76	3.69	4.98
HR age (95% CI)	0.87 (0.43-1.79)	Reference	1.12 (0.84-1.48)
HR all (95% CI)	0.80 (0.39-1.65)	Reference	1.14 (0.85-1.53)
Others			
Number of deaths	14	111	77
Mortality rate	4.83	3.62	4.46
HR age (95% CI)	1.58 (0.91-2.76)	Reference	1.01 (0.75-1.35)
HR all (95% CI)	1.50 (0.86-2.64)	Reference	1.04 (0.77-1.40)

Abbreviations: HR age, adjusted for age; HR all, adjusted for age, smoking status, drinking status, body mass index, education level, hypertension, diabetes, and high-density lipoprotein cholesterol; CI, confidence interval.

No significant statistical association was observed between soy intake and cardiovascular mortality in men and women in our study. In contrast, the JPHC study reported a significant inverse association between soy intake and the risk of mortality from ischemic cardiovascular diseases in women but not in men.[24] However, our data could not clearly demonstrate the reasons

Table 4. Crude Mortality Rates for 1000 Person-Years, Hazard Ratios, and Frequency of Soy or Soy Products Intake in Women.

	Frequency of Soy Intake				
	Rarely	1-2 Times/Month	1-2 Times/Wk	3-4 Times/wk	Almost Daily
Person-years	5833	19 361	26 289	17 783	11 363
All causes					
Number of deaths	29	71	119	80	55
Mortality rate	5.0	3.7	4.5	4.5	4.8
HR age (95% CI)	1.26 (0.84-1.90)	0.85 (0.63-1.13)	Reference	0.99 (0.75-1.32)	0.97 (0.71-1.34)
HR all (95% CI)	0.83 (0.51-1.36)	0.81 (0.52-1.28)	Reference	0.67 (0.41-1.08)	0.81 (0.51-1.30)
Cardiovascular					
Number of deaths	10	14	31	20	15
Mortality rate	1.7	0.7	1.2	1.1	1.3
HR age (95% CI)	1.65 (0.81-3.36)	0.66 (0.36-1.21)	Reference	0.99 (0.57-1.71)	0.94 (0.51-1.72)
HR all (95% CI)	0.59 (0.24-1.40)	0.59 (0.27-1.31)	Reference	0.38 (0.16-0.93)	0.62 (0.27-1.42)
Cancer					
Number of deaths	11	36	46	25	21
Mortality rate	1.9	1.9	1.7	1.4	1.8
HR age (95% CI)	1.19 (0.62-2.31)	1.09 (0.71-1.69)	Reference	0.79 (0.48-1.28)	0.97 (0.58-1.63)
HR all (95% CI)	1.02 (0.45-2.32)	1.03 (0.48-2.21)	Reference	1.03 (0.47-2.26)	0.77 (0.34-1.73)
Others					
Number of deaths	8	20	40	34	19
Mortality rate	1.4	1.0	1.5	1.9	1.7
HR age (95% CI)	1.06 (0.49-2.26)	0.72 (0.42-1.22)	Reference	1.26 (0.80-1.99)	1.01 (0.58-1.74)
HR all (95% CI)	0.91 (0.37-2.19)	0.81 (0.36-1.84)	Reference	0.61 (0.25-1.47)	1.09 (0.48-2.50)

	Frequency of Soy Products Intake		
	Less Than 1-2 Times Per Month	1-4 Times Per Week	Almost Every Day
Person-years	2877	42 177	35 575
All causes			
Number of deaths	12	172	170
Mortality rate	4.17	4.08	4.78
HR age (95% CI)	0.89 (0.49-1.59)	Reference	1.08 (0.88-1.35)
HR all (95% CI)	0.76 (0.39-1.49)	Reference	1.06 (0.85-1.33)
Cardiovascular			
Number of deaths	5	47	42
Mortality rate	1.73	1.11	1.18
HR age (95% CI)	1.31 (0.52-3.29)	Reference	0.98 (0.66-1.51)
HR all (95% CI)	1.28 (0.46-3.59)	Reference	1.03 (0.66-1.61)
Cancer			
Number of deaths	4	68	67
Mortality rate	1.39	1.61	1.88
HR age (95% CI)	0.77 (0.28-2.12)	Reference	1.08 (0.77-1.52)
HR all (95% CI)	0.43 (0.11-1.76)	Reference	1.10 (0.77-1.58)
Others			
Number of deaths	3	57	61
Mortality rate	1.04	1.35	1.71
HR age (95% CI)	0.67 (0.21-2.13)	Reference	1.19 (0.83-1.71)
HR all (95% CI)	0.78 (0.24-2.45)	Reference	1.06 (0.71-1.57)

Abbreviations: HR age, adjusted for age; HR all, adjusted for age, smoking status, drinking status, body mass index, education level, hypertension, diabetes, high-density lipoprotein cholesterol, and menopause; CI, confidence interval.

for the difference. Soy isoflavones can act as antioxidants, reducing the formation of oxidized lipoproteins, such as low-density lipoprotein.[25] Several articles have reported a reduced potential for oxidation in serum in those who consume soy proteins.[25,26] A meta-analysis has suggested that the incidence of ischemic heart disease increases after menopause, and this increase is partly

associated with a decrease in the antiatherosclerotic action of estrogen.[6] In a cohort study on the incidence of stroke in Japanese individuals, the risk of stroke differed between men and women,[27] and in premenopausal women, the risk of stroke was low, possibly because of the protective effect of estrogen.[28,29] In the present study, menopausal women had a higher frequency of soy intake than premenopausal women (Tables 1 and 2). As a result, the potentially increased risk of atherosclerotic cardiovascular disease in postmenopausal women could be balanced by a higher intake of soy.

The present study has some limitations. First, although the study participants were selected from a population-based health check-up system, they were not randomly selected. The proportion of participants treated for hypertension, diabetes mellitus, or dyslipidemia was lower than that in a national health and nutrition examination survey.[30] Therefore, the participants were somewhat healthier than the general population. Second, the presence of residual confounding factors could not be ruled out, although several approaches such as exclusion, multivariate modeling, and stratification were used to control for the effects of potential confounders. The participants who frequently consumed soy may have had potentially healthier behavior, such as a greater intake of healthy foods. However, consistencies in all analyses suggested that confounding, apart from food intake, was not a feature in the present analysis. Finally, detailed questions regarding the quantity of soy consumed were not asked in the surveys included in the present study. An earlier study suggested a significant inverse relationship between the frequency and quantity of soy intake and cerebral and myocardial infarctions.[6] However, because the present study was concerned only with the frequency of soy intake, there may have been a disparity between the frequency and quantity of soy intake.

Conclusions

In the present study, the frequency of soy intake in men was associated with all-cause mortality and cancer mortality. An increase in all-cause mortality and cancer mortality was found in male participants who reported rarely consuming soy. Cancer mortality also increased when soy was consumed almost daily. In women, daily intake of soy did not lower the risk of all-cause mortality and was not associated with a reduction in the risk of cardiovascular mortality or cancer mortality. These results suggest that a moderate frequency of soy intake could reduce mortality risk in the Japanese male population. Our results, however, were unable to demonstrate why those consuming soy most frequently failed to receive a protective effect of soy against cancer mortality. Further epidemiological studies are needed to clarify the health benefits of soy.

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Original Article

Breakfast Skipping is Positively Associated With Incidence of Type 2 Diabetes Mellitus: Evidence From the Aichi Workers' Cohort Study

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ABSTRACT

Background: Skipping breakfast has been suspected as a risk factor for type 2 diabetes (T2DM), but the associations are not entirely consistent across ethnicities or sexes, and the issue has not been adequately addressed in the Japanese population.

Methods: We followed 4631 participants (3600 men and 1031 women) in a work-site cohort of participants aged 35–66 years in 2002 through 2011 for T2DM development. Frequency of eating breakfast was self-reported and was subsequently dichotomized to breakfast skippers, who eat breakfast 3–5 times/week or less, and to eaters. Cox proportional hazards models were used to adjust for potential confounding factors, including dietary factors, smoking and other lifestyles, body mass index (BMI), and fasting blood glucose (FBG) at baseline.

Results: During 8.9 years of follow-up, 285 T2DM cases (231 men and 54 women) developed. Compared to participants who reported eating breakfast every day, maximally-adjusted hazard ratios and 95% confidence intervals (CI) of those with the frequency of almost every day and 3–5, 1–2, and 0 days/week were: 1.06 (95% CI, 0.73–1.53), 2.07 (95% CI, 1.20–3.56), 1.37 (95% CI, 0.82–2.29), and 2.12 (95% CI, 1.19–3.76), respectively. In a dichotomized analysis, breakfast skipping was positively associated with T2DM incidence (maximally-adjusted hazard ratio 1.73; 95% CI, 1.24–2.42). The positive associations were found in both men and women, current and non-current smokers, normal weight and overweight (BMI $\geq$25 kg/m^2), and normal glycemic status and impaired fasting glycemic status (FBG 110 to <126 mg/dL) individuals at baseline (*P*s for interaction all >0.05).

Conclusions: The present study in middle-aged Japanese men and women suggests that skipping breakfast may increase the risk of T2DM independent of lifestyles and baseline levels of BMI and FBG.

Key words: breakfast; diabetes mellitus; cohort study; Japan

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major cause of morbidity and mortality globally.[1] Indeed, the prevalence of diabetes in Japanese aged 40–69 was reported to be as high as 10.2% in male workers and 4.7% in female workers in large-scale companies in 2008–2010 and 15.0% in men and 8.0% in women in the National Health and Nutrition Survey in 2011.[2,3] Diabetes was also the 14th highest cause of disability-adjusted life years (DALYs) in Japan in 2010.[4] It is estimated that diabetes prevalence will remain roughly stable in the next 20 years.[5]

At the same time, skipping breakfast has been suggested to be associated with the incidence of several diseases or conditions, such as obesity,[6] insulin insensitivity,[7] cardiovascular diseases,[8] and T2DM.[9–11] However, the associations of breakfast skipping with T2DM are not entirely consistent across different ethnicities or sexes, and

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the issue has not been adequately addressed in the Japanese population. Furthermore, breakfast skipping is becoming common in Japan, as the 2013 National Health and Nutrition Survey indicated that 14.4% men and 11.1% women start their day without having breakfast.[3] Since breakfast eating habit is modifiable, examining the possible causal relationship has public health significance in the prevention of T2DM.

Therefore, we designed the present study to examine the association between breakfast skipping and the incidence of T2DM in a large-scale prospective cohort of middle-aged Japanese men and women, and to find out whether the association would be independent of diet and lifestyles, body mass index (BMI), and fasting blood glucose (FBG) levels at baseline.

METHODS

Study population

We used data obtained from the Aichi workers' cohort study. The cohort included 6648 Japanese civil servants in Aichi prefecture, an urban and suburban area located in central Japan. Participants were between 35 and 66 years old at recruitment in 2002. Most workers in the cohort were engaged in clerical work. Police officers, firefighters, and public school teachers were not included in the cohort, but health professionals working in prefectural hospitals were included. The baseline survey conducted in 2002 included a self-administered questionnaire concerning their lifestyle and medical history, as well as a health checkup. Written informed consent was obtained in advance separately for the lifestyle questionnaire and the use of annual health checkup data.

We excluded the following participants: (1) those who did not agree to our use of the annual health checkup results ($n = 1045$); (2) those with missing information for frequency of eating breakfast ($n = 8$), energy intake ($n = 83$), and smoking status ($n = 48$); (3) prevalent cases of diabetes mellitus, defined as self-reported medication use ($n = 570$) or baseline glucose level ≥126 mg/dL ($n = 128$); and (4) those modifying their diet under physicians' or dieticians' suggestion ($n = 135$). After these exclusions, 4631 participants (3600 men and 1031 women) were available for the present analysis.

Participants were followed until the end of follow-up (March 31, 2011), censoring, or ascertainment of diabetes, whichever came first. Participants were censored when they retired, except for those who provided their postal address to the researchers. The number of participants who were censored due to retirement was 919. The study protocol was approved by the Ethics Review Committee of Nagoya University School of Medicine.

Ascertainment of incident T2DM

We ascertained incidence of T2DM by two methods. First, we defined the incidence as the year when FBG level reached ≥126 mg/dL. We arbitrarily set the date of onset as July 1st for the analysis, considering that the checkups were usually carried out from October to December and that T2DM would generally be a chronic state without a definite onset. Second, we utilized data from self-administered questionnaire surveys on medical history, which were conducted in 2004, 2007, and 2011. In the surveys, participants reported medical histories of T2DM and other pre-specified conditions. Those who had T2DM history provided information about the onset (year of diabetes diagnosis) as well as the name and address of their present or past physician. We obtained written consent to access participants' medical records via their specified physicians. We previously confirmed the accuracy (95%) of self-reports by reviewing the medical records from cases with written consent, and the details of the validation study have been reported elsewhere.[12] Health checkups were provided annually during their employment. After retirement, participants were followed only by the questionnaire. Health checkup results of retired participants were not systematically collected.

Frequency of eating breakfast

In this study, we assessed frequency of eating breakfast using a self-administered questionnaire. Breakfast eating frequency was assessed using the following five categories: every day, almost every day with occasional skips, 3–5 days/week, 1–2 days/week, and none. We later reclassified the participants into two groups as breakfast eaters (those who reported their breakfast eating frequency as every day and almost every day with occasional skips) and breakfast skippers (those who classified themselves in the breakfast eating categories of 3–5 days/week, 1–2 days/week, and none). The reproducibility (Spearman's correlation coefficient) of the breakfast eating frequency question over a 9-month period has been reported to be 0.62.[13] In addition, 752 out of the present 4631 participants also reported breakfast eating frequency 5 years after the baseline (in 2007). The agreement between these two surveys was still fair (Spearman's correlation coefficient: 0.55).

Other dietary factors

The self-administered brief-type dietary history questionnaire (BDHQ) was used for the assessment of diet, including intakes of nutrients, alcohol, and total energy.[14,15] Intakes of fish, fruits, vegetables, whole-grain cereals, coffee, sugar-sweetened beverages, and snacks obtained by the BDHQ were adjusted for total energy intake by the nutrient density method.[14] Eating speed was self-reported in the BDHQ as very fast, relatively fast, medium, relatively slow, and slow. The last two categories were combined in the analysis. In addition to the information obtained by the BDHQ, information about participants' habit of eating to satiety was also obtained.

Anthropometric measurements and biochemical analysis

Height was measured to the nearest 0.1 cm with participants standing upright against a stadiometer without shoes. Body weight was measured to the nearest 0.1 kg with the participants in typical indoor clothing. BMI was calculated as weight (kg) divided by the square of height (m). Venous blood samples were drawn after the participants fasted for 8 h or more (or overnight), and serum samples were frozen at −80°C until the biochemical assay. Blood glucose was enzymatically determined by the hexokinase method. Insulin concentration was measured by solid-phase radioimmunoassay (RIABEAD II; Dinabot Co., Ltd., Chiba, Japan).

Other variables

Smoking status was classified into three categories (current, former, and never). The number of days engaged in leisure-time physical activity for 60 minutes or more was self-reported and classified into two categories: ≥3 days/week or <3 days/week. Work-time physical activity was assessed by the question "Are you engaged in physical labor?" and classified into two categories (yes or no). Work schedule was classified into four categories: with shiftwork including night shifts, with shiftwork but without night shifts, without shiftwork but with night work, and without shiftwork or night work. Sleep duration was classified into two categories: <7 h or ≥7 h. Strength of perceived stress was self-reported by the following four categories: very much, much, ordinary, and little. Family history of diabetes among first-degree relatives was self-reported and used as a dichotomized variable (yes or no) in the analysis.

Statistical analysis

FBG values were log-transformed to approximately normalize their distribution prior to the analyses and are presented as geometric means and their 95% confidence intervals (CIs). Other continuous variables were summarized as means and standard deviations (SD), while percentages were used for categorical variables. One-way analysis of variance or χ^2 tests were used, as appropriate, to compare baseline characteristics of breakfast eaters and breakfast skippers.

Multivariable adjusted Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% CIs of the risk of T2DM according to breakfast eating frequency and in breakfast skippers relative to breakfast eaters. Model 1 was adjusted for age, sex, total energy intake, smoking status, alcohol consumption, leisure-time physical activity, work-time physical activity, family history of diabetes mellitus, eating speed, perceived stress, sleep duration, work schedule, habit of eating to satiety, and intakes of fruits and vegetables, fish, whole-grain cereals, coffee, sugar-sweetened beverages and snacks. In model 2, BMI (continuous) was included in addition to all the variables in model 1. We adjusted for FBG (continuous) in model 3 together with the variables in model 2.

We then performed the above analyses stratified by sex, smoking status (current, never, or former) and baseline values of BMI (<25 kg/m^2 or ≥25 kg/m^2) and FBG (<110 mg/dL or ≥110 mg/dL). We also conducted sensitivity analyses by excluding incident cases who were followed up for less than 3 years (n = 119) and those who were night shift workers (n = 53). Furthermore, we carried out an analysis by censoring all the participants at the age of 60 (retirement age) because not all the retired participants were followed up (n of participants who were followed up after retirement = 881).

We also performed an analysis by updating breakfast skipping information with the data obtained after 5 years from baseline when available (approximately 70%) using time-dependent Cox regression model. For those who did not have such information, we carried forward the 2002 frequency.

Another ancillary analysis changing the outcome to impaired fasting glucose (IFG) or diabetes defined as FBG ≥110 mg/dL was performed in an attempt to examine similarity and discrepancy of our findings to a prior study that used such outcome.[16]

All statistical analyses were conducted with IBM SPSS Statistics for Windows, Version 22.0 software (IBM Corp, Armonk, NY, USA). All tests were two-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Out of the 4631 participants included in our analysis, 90.4% were breakfast eaters. Compared with breakfast eaters, breakfast skippers seem to have worse lifestyles. For example, breakfast skippers were more likely to be current smokers, consumed more alcohol and sugar-sweetened beverages, and had less intakes of fruits and vegetables (all $P < 0.05$) (Table 1).

During a median of 8.9 years of follow-up, 285 cases of T2DM (231 men and 54 women) developed (crude incidence rate: 8.2 per 1000 person-years). Participants who reported eating breakfast 3–5 days/week, 1–2 days/week, and 0 days/week had higher T2DM incidence than those who consumed breakfast every day (model 3 HRs ranging from 1.37 to 2.12) (Table 2). However, the point estimates fluctuated and were not always statistically significant, and there was no apparent positive trend in the T2DM incidence according to the number of days breakfast was skipped. In addition, T2DM incidence of those who skipped breakfast only occasionally was not higher than every day eaters (model 3 HR 1.06). In the subsequent dichotomized analysis, T2DM incidence of breakfast skippers was significantly higher than that of breakfast eaters (crude incidence rate: 13.9/1000 person-years vs 7.5/1000 person-years; model 3 HR 1.73). The positive associations between breakfast skipping and T2DM were similar in both men and women, current and non-current smokers, and normal weight and overweight individuals, as well as those with normal glycemic status and those with

Table 1. Participants' demographic, lifestyle, dietary habits, and metabolic risk factor characteristics according to breakfast consumption status at baseline, Aichi, 2002

	Breakfast eaters[a]	Breakfast skippers[b]	*P* value[c]
n, %	4188, 90.4	443, 9.6	
Men, %	78.2	73.8	0.04
Age, year	47.8 (7.1)	46.0 (6.8)	<0.001
Body mass index, kg/m^2	22.9 (2.8)	22.9 (3.0)	0.78
Smoking status, %			
Current	26.7	44.9	<0.001
Former	23.4	14.2	
Never	49.9	40.9	
Leisure-time physical activity, %[d]			
≥3 days/week	75.5	84.2	<0.001
<3 days/week	16.0	9.5	
Work-time physical activity, yes, %	5.3	7.7	<0.01
Family history of diabetes mellitus, yes, %	14.8	17.4	0.14
Fasting blood glucose, mg/dL[e]	92.3 (92.0–92.6)	92.5 (91.5–93.4)	0.74
Perceived stress, %[d]			
Very much	11.0	13.8	0.02
Much	40.1	39.7	
Ordinary	43.8	39.1	
Little	4.9	6.8	
Sleep duration, %[d]			
<7 hours/day	52.9	61.6	<0.001
≥7 hours/day	45.5	35.7	
Work schedule, %[d]			
Without shift work or night shifts	84.6	78.3	<0.001
With shift work but without night shifts	1.9	1.4	
Without shift work but with night shifts	6.8	7.9	
With shift work including night shifts	4.9	11.3	
Total energy intake, kcal/day	1942 (538)	1740 (553)	<0.001
Alcohol consumption, g/day	13.6 (19.2)	18.1 (26.4)	<0.001
Eating speed, %[d]			
Very fast	11.4	12.9	0.67
Relatively fast	35.8	37.9	
Medium	38.5	35.2	
Slow	12.6	12.4	
Satiation eater, %	61.2	59.1	0.24
Fruits and vegetables intake, g/1000 kcal/day	142.3 (67.0)	118.7 (61.2)	<0.001
Fish intake, g/1000 kcal/day	84.1 (41.9)	84.8 (39.4)	0.74
Frequency of whole-grain cereals intake, %			
Always	8.5	5.7	0.10
Sometimes	9.2	8.1	
Rarely	14.9	12.6	
No	67.4	73.6	
Frequency of coffee intake, %[d]			
≥4 cups/day	6.6	10.6	0.02
2–3 cups/day	39.0	39.1	
1 cup/day	24.3	23.7	
<1 cup/day	28.8	25.1	
Frequency of sugar-sweetened beverages intake, %[d]			
≥1 serving/day	5.5	9.3	<0.001
4–6 servings/week	5.3	7.0	
1–3 servings/week	29.9	36.3	
Never or rarely	57.6	45.1	
Snack intake, yes, %	95.3	94.8	0.61

Values are reported as mean (standard deviation) or percentage.
[a]Breakfast eater was defined as those having breakfast eating frequency of 'every day or almost every day with occasional skips'.
[b]Breakfast skipper was defined as those having breakfast eating frequency of '3–5 days/week, 1–2 days/week, or none'.
[c]Obtained from ANOVA and Chi-square test for continuous and categorical variables, respectively.
[d]Proportions in each category do not add up to 100% when there were missing data.
[e]Geometric mean (95% confidence interval).

impaired fasting glycemic status at baseline (all interaction $P > 0.05$) (Table 3). Furthermore, similar associations were found in sensitivity analyses that excluded incident cases whose follow up periods in the cohort were less than 3 years (model 3 HR 1.94; 95% CI, 1.24–2.98) and those who were night shift workers (model 3 HR 1.91; 95% CI, 1.30–2.80). Also, the association by the analysis that censored all the participants who reached 60 years did not materially differ

Table 2. Incidence rates and hazard ratios of type 2 diabetes mellitus incidence according to breakfast consumption, Aichi, 2002–2011

	Frequency of eating breakfast				
	Every day	Almost every day with occasional skips	3–5 days/week	1–2 days/week	None
n of cases/*N*	204/3648	35/540	15/121	17/197	14/125
Crude incidence rate[a]	7.4	8.4	16.6	11.4	15.4
Crude HR (95% CI)	1 (reference)	1.13 (0.79–1.62)	2.25 (1.33–3.80)	1.54 (0.94–2.53)	2.08 (1.21–3.58)
Model 1[b] HR (95% CI)		1.11 (0.77–1.60)	1.93 (1.12–3.33)	1.51 (0.91–2.51)	1.96 (1.11–3.45)
Model 2[c] HR (95% CI)		1.11 (0.77–1.60)	1.97 (1.14–3.39)	1.46 (0.87–2.44)	2.09 (1.18–3.68)
Model 3[d] HR (95% CI)		1.06 (0.73–1.53)	2.07 (1.20–3.56)	1.37 (0.82–2.29)	2.12 (1.19–3.76)

	Breakfast eaters[e]	Breakfast skippers[f]
n of cases/*N*	239/4188	46/443
Crude incidence rate	7.5	13.9
Crude HR (95% CI)	1 (reference)	1.85 (1.35–2.54)
Model 1[b] HR (95% CI)		1.72 (1.23–2.40)
Model 2[c] HR (95% CI)		1.74 (1.24–2.43)
Model 3[d] HR (95% CI)		1.73 (1.24–2.42)

CI, confidence interval; HR, hazard ratio; *n*, number; *N*, number of participants.
[a]Crude incidence rate (per 1000 person-years).
[b]Model 1: Adjusted for age, sex, total energy intake, smoking status, alcohol consumption, leisure-time physical activity, work-time physical activity, family history of diabetes mellitus, eating speed, perceived stress, sleep duration, work schedule, satiation eater, fruits and vegetables intake, fish intake, and intake frequencies of whole-grain cereals, coffee, sugar-sweetened beverages, and snacks.
[c]Model 2: Model 1 + body mass index.
[d]Model 3: Model 2 + fasting blood glucose (Log-transformed).
[e]Breakfast eater was defined as those having breakfast eating frequency of 'every day or almost every day with occasional skips'.
[f]Breakfast skipper was defined as those having breakfast eating frequency of '3–5 days/week, 1–2 days/week, or none'.

Table 3. Incidence rates and hazard ratios of type 2 diabetes mellitus according to breakfast consumption stratified by sex, body mass index, fasting blood glucose, and smoking status at baseline, Aichi, 2002–2011

	Breakfast eaters[a]	Breakfast skippers[b]	Breakfast eaters[a]	Breakfast skippers[b]
Sex	Men		Women	
n of cases/*N*	197/3273	34/327	42/915	12/116
Crude incidence rate[c]	7.8	13.8	6.4	14.4
Model 3[d] HR (95% CI)	1 (reference)	1.54 (1.05–2.28)	1 (reference)	2.29 (1.05–5.02)
Smoking status	Current smoker		Never or former smoker	
n of cases/*N*	85/1118	22/199	154/3070	24/244
Crude incidence rate	10.2	14.9	6.6	13.1
Model 3[d] HR (95% CI)	1 (reference)	1.41 (0.85–2.33)	1 (reference)	2.17 (1.36–3.46)
Body mass index	<25 kg/m^2		≥25 kg/m^2	
n of cases/*N*	148/3298	28/332	91/890	18/111
Crude incidence rate	5.9	11.0	13.6	23.6
Model 3[d] HR (95% CI)	1 (reference)	1.67 (1.08–2.58)	1 (reference)	1.98 (1.13–3.47)
Fasting blood glucose	<110 mg/dL		≥110 mg/dL	
n of cases/*N*	177/3943	36/414	62/245	10/29
Crude incidence rate	5.9	11.5	41.4	55.1
Model 3[d] HR (95% CI)	1 (reference)	1.80 (1.23–2.64)	1 (reference)	1.62 (0.75–3.52)

CI, confidence interval; HR, hazard ratio; *n*, number; *N*, number of participants.
[a]Breakfast eater was defined as those having breakfast eating frequency of 'every day or almost every day with occasional skips'.
[b]Breakfast skipper was defined as those having breakfast eating frequency of '3–5 days/week, 1–2 days/week, or none'.
[c]Crude incidence rate (per 1000 person-years).
[d]Model 3 was adjusted for age, sex (if appropriate), total energy intake, smoking status (if appropriate), alcohol consumption, leisure-time physical activity, work-time physical activity, family history of diabetes mellitus, eating speed, perceived stress, sleep duration, work schedule, satiation eater, fruits and vegetables intake, fish intake, and intake frequencies of whole-grain cereals, coffee, sugar-sweetened beverages, and snacks, as well as body mass index (continuous) and fasting blood glucose (Log-transformed).

(model 3 HR 1.71; 95% CI, 1.21–1.49). The analysis updating breakfast skipping information also yielded similar results (model 3 HR 1.66; 95% CI, 1.19–2.32). In another ancillary analysis that used IFG or T2DM as the outcome among participants whose baseline FBG <110 mg/dL (3371 men and 986 women), skipping breakfast was also significantly associated with higher incidence of IFG or T2DM (*n* of incident cases: 707; model 3 HR 1.29; 95% CI, 1.02–1.63).

DISCUSSION

In the present study, we found that breakfast skipping was positively associated with T2DM incidence in middle-aged Japanese men and women, after adjustment for a number of potential confounding variables, including baseline BMI and FBG levels. We confirmed the associations in both men and women, and in individuals with or without overweight or IFG at baseline. Although the association in current smokers was not statistically significant, formal test of interaction did not suggest any statistically significant difference in the association between current and non-current smokers. The positive association between breakfast skipping and T2DM observed in our study is in line with previous studies conducted in the United States.[9–11] We extended the finding to a Japanese sample and confirmed the associations in several subgroups.

The positive association between breakfast skipping and the risk of T2DM shown in our study is also roughly in line with a previous study in Japan,[16] although the study only found the association in women, did not adjust for important lifestyle variables or perform detailed stratified analyses, and used IFG as the outcome. Our ancillary analysis that employed IFG or T2DM as the outcome found a weaker but similar association as the original analysis.

In the present study, we did not find a dose-response association between breakfast eating (skipping) frequency and T2DM incidence. This is not consistent with a finding from the CARDIA Study (average baseline age: 32 years), which reported a stepwise inverse association between breakfast eating frequency and T2DM incidence in white men and women and in black men but not black women. The reason for the discrepancy is not clear. Other studies conducted in the United States did not specifically address the issue of dose-response. Also, caution is required in interpreting results, since the definition of skipping breakfast differs by studies.[10,11]

Since previous studies in Japan reported associations between breakfast skipping and higher BMI[6,17] as well as regular smoking, we attempted to examine the association precisely by stratifying the analyses by these variables, and we found that the associations were similar across the strata. Nonetheless, the relatively stronger effect estimates observed among those in the higher baseline BMI categories may somehow corroborate the belief that BMI could influence participants' dietary habits, including breakfast eating behavior, and hence may modify its association with T2DM.[6,17] However, whether or not this was really due to the influence of participants' prior knowledge of their BMI and FBG needs further investigation.

We speculated a few mechanisms by which breakfast skipping could potentially cause T2DM. First, it has been reported that after-lunch postprandial glucose and insulin levels were significantly higher in participants who skipped breakfast than those who consumed breakfast.[18] Similarly, omitting breakfast has been reported to impair postprandial insulin sensitivity.[7] Since the level of 1,5-anhydroglucitol, an indicator for short-term hyperglycemia and glycemic excursions, was significantly associated with the development of diabetes independent of HbA1c levels, metabolic alterations induced by breakfast skipping may predispose individuals to diabetes.[19] Second, skipping breakfast could mean having infrequent larger meals. Total energy intakes in the present study were 1942 and 1740 kcal/day in breakfast eaters and breakfast skippers, respectively. We could guess that breakfast skippers had more energy intake per serving, which means that they had larger serving sizes than breakfast eaters. This may also be associated with future diabetes incidence through greater postprandial glucose and insulin responses. The relationship between breakfast skipping and T2DM observed in our study could also be due to residual confounding of other lifestyles.[17] Although we have adjusted for a number of lifestyles, breakfast skippers may have other lifestyle and behavior characteristics that may cause T2DM.

Our study has several other limitations. First, although the BDHQ had fair reproducibility over 9 months as well as 5 years, breakfast consumption was self-reported and subject to a subjective interpretation of what constitutes a breakfast and duration of fasting before the meal. However, the possible information bias as a result would be non-differential and is not expected to influence our findings. Indeed, no material difference in our finding was seen after excluding night shift workers who may have the habit of taking early morning snacks.[20,21] Second, there was no information on the nutrient composition of the breakfast consumed, and we could not assess the effect of quality of breakfast on the association between breakfast consumption and T2DM. Since breakfast cereal intake was inversely associated with T2DM incidence,[22] the association might have been different if we had the information. Third, we ascertained T2DM incidence by a single identification of diabetic FBG level. Although this definition is commonly employed in epidemiologic studies, additional measurement of HbA1c or oral glucose tolerance test results should ideally be used. We also utilized self-reports for T2DM ascertainment, which would have high specificity but relatively low sensitivity.[23] Finally, the observational nature of the present study would prevent any definitive statement about causality. Although our study participants

were relatively homogeneous middle-aged Japanese civil servants with similar demographic, socioeconomic, and environmental backgrounds, there may still be unknown or uncontrolled confounding. While a long-term randomized controlled trial would be difficult to conduct, well-designed cohort studies in other settings may be useful, since findings on the issue are still scarce and have not always been consistent.

In summary, our findings indicate that skipping breakfast increased the risk of T2DM in middle-aged Japanese workers, and the association was independent of several dietary and lifestyle factors, as well as baseline levels of BMI and FBG. Public health messages promoting the benefits of eating breakfast could be distributed in civil service institutions in Japan and the public at large.

ONLINE ONLY MATERIAL

Abstract in Japanese.

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Conflicts of interest: None declared.

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Original Article

Smoking and Diabetes: Is the Association Mediated by Adiponectin, Leptin, or C-reactive Protein?

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ABSTRACT

Background: Although the association between cigarette smoking and risk of type 2 diabetes is well established, its mechanisms are yet to be clarified. This study examined the possible mediating effects of adiponectin, leptin, and C-reactive protein (CRP) concentrations on the smoking-diabetes association.

Methods: Between 2002 and 2011, we followed 3338 Japanese workers, aged 35–66 years, who were enrolled in the second Aichi workers' cohort study. We used multivariable-adjusted Cox regression models to determine the hazard ratios and respective 95% confidence intervals (CIs) of the association between smoking status and risk of diabetes. A multiple mediation model with bootstrapping was used to estimate the magnitude and the respective bias-corrected (BC) 95% CIs of the indirect effects of smoking on diabetes through the three biomarkers.

Results: Relative to never smokers, the risk of diabetes was significantly elevated in current (hazard ratio 1.75, 95% CI 1.25–2.46) and ex-smokers (hazard ratio 1.54, 95% CI 1.07–2.22). The indirect effects of smoking on diabetes through adiponectin levels were statistically significant among light (point estimate 0.033, BC 95% CI 0.005–0.082), moderate (point estimate 0.044, BC 95% CI 0.010–0.094), and heavy smokers (point estimate 0.054, BC 95% CI 0.013–0.113). In contrast, neither the indirect effects of smoking on diabetes through leptin nor CRP levels were significant, as the corresponding BC 95% CIs included zero.

Conclusions: In our analysis, adiponectin concentration appeared to partially mediate the effect of smoking on diabetes, while leptin and CRP levels did not.

Key words: adiponectin; C-reactive protein; leptin; mediation analysis; smoking; type 2 diabetes mellitus

INTRODUCTION

Cigarette smoking is independently associated with incidence of type 2 diabetes mellitus (DM).[1,2] Although details behind the mechanism of this association are not yet fully understood, promotion of central obesity, hypercortisolaemia, nicotine-induced impaired beta-cell function, and elevations in inflammatory markers and oxidative stress caused by smoking are suspected.[2,3]

Adiponectin and leptin are the most abundant adipocytokines produced by adipocytes and have anti- and pro-inflammatory properties, respectively.[4,5] Smoking may cause a decrease in adiponectin levels,[6–10] and decreased adiponectin levels have been consistently associated with DM incidence.[11–14] With regard to leptin, previous studies on its association with smoking have often yielded conflicting results: reports of decrease,[15–18] no effect,[19] or increase[20,21] in the leptin levels of smokers are all available. Similarly, reports on the association between serum leptin levels and DM incidence have been inconsistent: significant associations have been documented in some[22–24] but not all[25–27] studies.

C-reactive protein (CRP), an acute-phase reactant produced primarily in the liver, has been shown to be a sensitive, systemic biomarker of inflammation.[28] Several studies have

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consistently reported a positive association between smoking and CRP levels.[29–33] A number of prospective studies have also described the association between circulating CRP levels and DM incidence, with some demonstrating an independently positive association,[34–36] while others show no association.[25,26,37,38]

We hypothesized that serum levels of adiponectin, leptin, and CRP could potentially mediate the association between smoking and incidence of DM. We focused on these three biomarkers because they are the most common biomarkers reported in the literature in relation to smoking and DM. We evaluated our hypothesis using data obtained from the second Aichi workers' cohort study.[39]

METHODS

Study population

The second Aichi workers' cohort study is an ongoing study on cardiovascular diseases among civil servants aged 35 to 66 years in Aichi Prefecture, located in central Japan. Baseline information was collected from 6648 individuals in 2002 through self-administered questionnaires and mandatory annual health check-ups provided by the worksites of study subjects. We excluded subjects with missing values for the following variables: smoking status ($n = 100$); adiponectin, CRP, or leptin levels ($n = 2613$); and other covariates ($n = 332$). Prevalent cases of DM ($n = 265$), diagnosed by self-reported medication use or baseline fasting glucose level ≥126 mg/dL, were also excluded, leaving 3338 subjects for the present analysis. The excluded subjects were not significantly different from those included in the analyses with respect to distribution of variables, including sex, age, smoking status, and DM incidence.

Subjects were followed until they retired. Workers who were reemployed after their retirement age of 60 years were kept in the cohort until they re-retired. Those who retired and were not reemployed were contacted by mail. However, those who did not provide their mailing address were censored at the time of retirement. There were no significant differences between retired subjects who provided mailing addresses and those who did not with regard to their smoking status, body mass index (BMI), and DM incidence. The study protocol was approved by the Ethics Review Committee of Nagoya University School of Medicine, Nagoya, Japan.

Measurement of exposures, confounders, and outcomes

Smoking metrics at baseline

Information on smoking status was acquired through a self-administered questionnaire. Subjects initially responded to an item that classified them as never, ex-, or current smokers. Ex-smokers were defined as those who do not currently smoke cigarettes but had previously smoked for at least a year. Both current and ex-smokers were asked to report the average number of cigarettes they smoke or had smoked per day and the age at which they started smoking. Ex-smokers were also asked to specify the age at which they quit smoking. Duration of smoking for ex-smokers at baseline was calculated in years by subtracting age at the time of cessation from the baseline age. If they had quit more than one time, the subjects were asked to specify the longest duration for which they had abstained from smoking. We categorized current smokers as light (1–19 cigarettes per day), moderate (20–29 cigarettes per day), and heavy smokers (≥30 cigarettes per day). Assuming 20 cigarettes per pack, pack-years of smoking were estimated using the formula, "(cigarettes per day/20) × years smoked".

Adiponectin, leptin, and CRP

Venous blood samples were drawn from each subject after at least 8 h of fasting. Serum samples were stored at −80°C until biochemical assay. Adiponectin concentration was determined in a commercial laboratory using an enzyme-linked immunosorbent assay (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), for which the laboratory reports intra-assay coefficients of variation of 6.0% to 8.6%. Leptin concentrations were measured via radioimmunoassay (Human Leptin RIA Kit; Linco Research, Inc., St. Charles, MO, USA) in a commercial laboratory. The detection limit of the leptin assay was 0.5 ng/mL, and the inter-assay coefficients of variation were 1.79% and 1.75% for low- and high-concentration controls, respectively. High-sensitivity CRP was measured by latex nephelometry (BNII; Siemens AG, Erlangen, Germany). The assay was sensitive enough to detect 0.02 mg/L of CRP with an inter-assay coefficient of variation of <4.0%.

Fasting blood glucose and other laboratory measurements

Fasting blood glucose was enzymatically determined via the hexokinase method. Insulin concentration was measured by solid-phase radioimmunoassay (RIABEAD II; Dinabot Co., Ltd., Chiba, Japan). Insulin resistance was evaluated with a homeostasis model assessment (HOMA2-IR) using the University of Oxford Diabetes Trials Unit's HOMA Calculator software (downloaded at http://www.dtu.ox.ac.uk). Total cholesterol and triglycerides were also measured via enzymatic methods, while high-density lipoprotein cholesterol (HDL-C) was determined via the phosphotungstate method.

Other Covariates

Dietary habits during the preceding month were assessed using a validated self-administered brief diet history questionnaire. Total energy and nutrient intakes were estimated using an ad hoc computer algorithm developed for nutrient calculation of the brief diet history questionnaire, with reference to the standard tables of food composition in Japan.[40] Alcohol consumption was calculated by multiplying weekly frequency and the amount drunk on each occasion, with values then converted into grams of ethanol per day. Physically active individuals were defined as those who

self-reported to be engaged in a moderate or vigorous leisure-time exercise for a total of 60 minutes or more for more than a day per month. We also assessed self-reported family history of DM among the subjects' first-degree relatives.

Ascertainment of incident diabetes

Incident cases of DM were ascertained using two sources of data: annual mandatory health checkups at work places and questionnaire surveys. As the health checkups were usually carried out from September to December, we defined the date of DM onset as July 1 of the year when fasting glucose level first went beyond 126 mg/dL. The self-administered questionnaire surveys were conducted between 2004 and 2011, in which participants reported their detailed medical histories of various conditions, including DM. Whenever appropriate, participants revealed the year of DM diagnosis as well as the name and address of their present or past physician. Written consent for our access to the participants' medical records from their physicians was also obtained. For cases with consent, the accuracy of self-reports (95%) was previously confirmed by reviewing their medical records, and the details of the validation study have been reported elsewhere.[39]

Statistical analyses

First, we generated descriptive statistics to determine whether there were differences in covariates among the five smoking categories (never, ex-, light, moderate, and heavy smokers). Variables with a skewed distribution (i.e., consumptions of alcohol and sugar, triglyceride, insulin, HOMA2-IR, adiponectin, leptin, and CRP levels) were log-transformed prior to further analyses to approximately normalize their distributions, and they were expressed as geometric means and 95% confidence intervals (CIs). Other continuous variables were expressed using means and standard deviations, while percentages were used for categorical variables. Analysis of variance (ANOVA) or χ^2 tests were used, as appropriate, to compare subjects' characteristics among the five smoking categories.

We used Cox proportional hazards regression models to estimate hazard ratios (HRs) and respective 95% CIs of the risk of DM among ex-smokers and the 3 categories of current smokers compared to never smokers. The models were adjusted for potential baseline confounders, including continuous variables of BMI, mean arterial pressure, sleep duration, total energy intake, alcohol consumption, sugar consumption, total cholesterol to HDL-C ratio, triglycerides, adiponectin, leptin, CRP, HOMA2-IR, as well as dichotomous variables of physical activity (yes/no) and family history of DM (yes/no). A stepwise backward elimination procedure was used to exclude from the final model those variables that did not substantially affect the results ($P > 0.1$). Age (continuous) and sex (dichotomous) were forced into the model at all times. The proportionality assumption was verified with log-log plots and by the use of Shoenfeld residuals. There was a tendency toward non-proportionality for the association between smoking status and incidence of DM. However, sensitivity analysis by logistic regression showed estimates similar to the Cox models, indicating no large violation of the proportionality assumption (results not shown).

Second, we conducted multiple mediation analysis using the PROCESS procedure for SPSS.[41] Standard path-analytic approaches[41–43] were followed to assess: 1) the effect of being an ex-(X_1), light (X_2), moderate (X_3), or heavy (X_4) smoker on DM incidence (Y) relative to never smokers (reference group)—*c* paths; 2) the differences between each smoking category and the reference group on the level of the proposed mediators (Ms), adiponectin (M_1), leptin (M_2), and CRP (M_3)—*a* paths; and 3) the association between the Ms and Y while statistically equating the groups on average on X and the other potential mediators in the model—*b* paths (Figure 1). Coefficients for *a* paths were estimated using ordinary least-squares regression, whereas logistic regression was used for the coefficients of the *b* and *c* paths. All of these analyses were adjusted for the covariates included in the Cox model mentioned above. Unstandardized coefficients (β) with their standard errors (SE) are reported for each model. The *c* paths represent the total effects of Xs on Y relative to the reference group unadjusted for the group differences in Ms. These can be apportioned into the direct effects of being in one X group on Y relative to the reference group adjusted for the group differences in Ms (*c'* paths) and the indirect effects through the Ms of being in one X group relative to the reference group on Y (estimated by the products of *a* and *b* paths—*ab*'s). Bias-corrected (BC) 95% CIs for the indirect effects were generated from 10 000 bootstrap samples, and statistical significance is indicated when the CI values do not cross zero. Bootstrapping is recommended for testing of indirect effects because it does not assume normality in sampling distribution.[43]

All statistical analyses were conducted using IBM SPSS Statistics for Windows software, Version 21.0 (IBM Corp, Armonk, NY, USA), and all tests were two-sided with the significance level set at $P < 0.05$.

RESULTS

Baseline characteristics

Characteristics of the study population across the five categories of smoking status at baseline are shown in Tables 1 and 2. Compared to never smokers, heavy smokers were characterized by lower serum levels of adipocytokines (adiponectin and leptin), higher levels of CRP, and more adverse cardio-metabolic risk factors. Incident diabetic cases were also more likely to be in the heavy smoker category.

Smoking status and risk of diabetes

Compared with the hazard ratio for subjects who never smoked, the age- and sex-adjusted hazard ratios of DM analyzed in aggregate were 1.62 (95% CI 1.13–2.34) among