strated the possibility that a dietary pattern with high intakes of fish, fruit, and vegetables and a low intake of meats may have a beneficial effect on BMD. Tucker and Okubo's observation, however, was not extended to look for associations between dietary patterns and fractures. There is no report investigating the relation of dietary patterns and fall-related fracture. Furthermore, the population studied by Okubo *et al.* was made up of pre-menopausal women. Though Tucker *et al.* studied an elderly population, the dietary habits of people from Western versus Asian countries are entirely different. As is well known, Japanese food is characterized by rice and soy bean products, and contains many types of fish, seafood and vegetables but only small amounts of meat or dairy products [15]. Therefore, in the present study, we examined the relationship between dietary patterns and fractures in elderly Japanese living in a suburb of Sendai, one of the largest cities in Northern Japan.

Methods

Study population

Our study population consisted of elderly subjects living in the Tsurugaya area of Sendai, the largest city of Tohoku (North-eastern) district in Japan. At the time of the study in 2002, there were 2730 people aged over 70 years living in the area. We invited all of these people to participate in a comprehensive geriatric assessment of medical status of whom 1178 agreed to participate and provided written informed consent for a baseline assessment. Of these 1178, we excluded 213 subjects who did not agree to the follow up survey, 77 with incomplete dietary data and 11 whose cognitive level was lower than 18 in the Mini Mental State Examination (MMSE) score [16]. Therefore, 877 participants whose medical status, activities of daily living (ADL), and life style, including dietary intakes, were assessed in July 2002 were followed up for their incidence of fall related fracture until the end of July 2006. Medical doctors (specializing in rehabilitation, exercise medicine and psychiatry), pharmacists, nurses, and kinesipathists assessed their baseline characteristics.

Assessment of dietary intake

The short version of a previously published self-administered food frequency questionnaire (FFQ) [17] was used for the present study. This included 75 food items with specified serving sizes that were described by natural portions or standard weight and volume measures of the servings commonly consumed in our study population. For each food item, participants indicated their mean frequency of consumption over the past year in terms of the specified serving size by checking 1 of the 7 frequency categories ranging from "almost never" to "2 or more times/d". Frequency data was converted to the gram intake as described previously [18]. The mean daily intake of nutrients was calculated using an ad hoc computer program developed to analyze the questionnaire. In the validity study of the present FFQ, the questionnaire provided close estimation of nutrients compared to the 3-day diet record [19].

Assessment of other variables

In addition to diet, we investigated the following factors related to fractures according to a WHO report [20]: age, gender, BMI calculated as weight (kg)/height (m) squared, MMSE as a measure of cognitive function, the medical outcome study questionnaire (MOS) [21] for ADL, smoking, past falls, past history of apoplexy, diabetes mellitus, osteoporosis, renal disease and cancer. Also we investigated the use of stabilizers, hypnotics, steroids and hormone replacement therapy (HRT). Anthropometric measurements i.e. height and body weight were recorded using a standard protocol. Alcohol consumption and use of supplements including calcium and multivitamins were assessed from the FFQ.

Diagnosis of fracture

The incidence and causes of any fractures were investigated based on insurance claim records from July 2002 until July 2006. Fracture data was available on all 877 participants including 39 subjects who had died in the follow-up period. All clinical records of patients with fractures were reviewed by a physician (R.N.). Cases involving traumatic fracture such as traffic accidents were included in the "Non fall-related fracture group".

Statistical analysis

Factor analysis was used to derive dietary patterns and to determine factor loadings for each of the 27 food subgroups. Factor analysis is a statistical method used to describe variability among observed variables in terms of fewer unobserved variables called factors [22]. Factors were rotated with varimax rotation to maintain uncorrelated factors and enhance interpretability. Dietary patterns were named according to the nature of the food groups loading highest for each of the factors. For each pattern and each participant, we calculated a factor score by summing the consumption of each food item weighted by its factor loading [18]. The subjects were divided into tertiles according to the factor score as follows: unconfirmed (the first tertile: T1), moderately confirmed (the second tertile: T2) and confirmed (the third tertile: T3) according to the factor score of each dietary pattern.

A simple logistical regression model was used to examine the relationships between the risk of fall-related fracture and general characteristics. Sample characteristics for T1, T2 and T3 in each dietary pattern were statistically analyzed using the parametric test. The Cox proportional hazards regression model was also used to examine the relationships between other variables mentioned above and the incidence of fall-related fractures with adjustment for age, gender [23], BMI [24] and energy intake. Hazard ratio (HR) and 95% CIs were calculated. The probabilities of being fracture free were estimated using the Kaplan-Meier product-limit method. Fracture free numbers were calculated from the date of enrolment to the date of fracture onset, or cut-off date for participants alive at the time of closure of the dataset. A significant difference was defined as $p < 0.05$. All statistical analyses were performed using the Statistical Analysis System 9.1 edition for WINDOWS (SAS Institute Inc, Cary, NC)

Ethics

The Institutional Review Board of Tohoku University Graduate School of Medicine approved the protocol of the study. Written informed consent was obtained from study participants. The study was not registered to any clinical trial registration websites because the study started in 2001 and the recruitment of participants was completed in 2002.

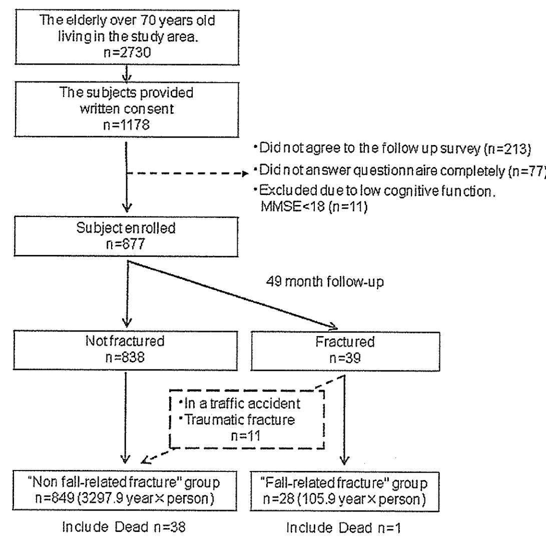

Figure 1 Study protocol. Of these 1178, we excluded 213 subjects who did not agree to the follow up survey, 77 with incomplete dietary data and 11 whose cognitive level was lower than 18 in the Mini Mental State Examination (MMSE) score. Therefore, 877 participants whose medical status, activities of daily living (ADL), and life style including dietary intakes were assessed in July 2002 were followed up until the end of July 2006.

Results

Study population

Of the 877 registered participants, 39 had suffered a fracture by the end of July 2006. Eleven participants had fractures due to traffic accident or other injuries. Therefore, we compared the remaining 28, who fractured due to a fall, to the other 849 participants who did not have a fall related fracture within our follow up period (Figure 1). Eleven persons who had fractures due to traffic accident or other injuries were included in the non fall-related fracture group. Their background, including age, height, weight, BMI, MMSE, MOS, energy intake, gender, history of stroke, diabetes, kidney disease, osteoporosis, cancer, use of tranquilizers, sleeping pills, steroids, supplements, HRT, smoking habit or alcohol, and falls in the previous 6 months were compared between the fall-related fracture and non fall-related fracture group. There were statistically significant differences in age (a mean of 82.3 years old in the fracture group and 79.1 years old in the non fall-related fracture group, $p = 0.01$) and smoking habit (a rate of 21.4% in the fall-related fracture group and 43.7% in the non fall-related fracture group, $p = 0.026$) (Table 1).

Dietary patterns identified

The factor-loading matrices are shown in Table 2. Factor 1 is loaded on a high consumption of vegetables, seaweeds, mushrooms, soy products and salt. Therefore, factor 1 was designated the Vegetable pattern. Factor 2 was designated the Meat pattern because it was loaded with a high consumption of meat (chicken, pork and beef), processed meat (ham, sausage, liver paste) and seafood (squid, octopus, shrimp, lobster and shellfish). Factor 3 was heavily loaded with rice and Miso soup intake. Also, this factor was mildly loaded with Natto (fermented soybean, a typical traditional soy product in East Asia). Therefore, we designated this factor as the Traditional Japanese pattern. The scree plots dropped on 2.5 after the third factor, factor 1 (eigenvalue 5.0) explaining 15.5% of the variability, factor 2 (3.0) explaining 7.3%, and factor 3 (2.8) explaining 7.2%.

Sample characteristics for T1, T2 and T3 in each dietary pattern are displayed in Additional file 1, Table S3. ADL (MOS), past history of diabetes and use of supplements showed a significant trend in the Vegetable pattern. Age, height, weight, ADL, % of male, past history of osteoporosis (decreased) and cancers (decreased), smoking and drinking habits showed significant trends in the Meat pattern. Finally, Height, weight, ADL (MOS), percentage of male, past history of stroke, diabetes, osteoporosis (decreased) and cancers (decreased), smoking and drinking habits showed significant trends in the Traditional Japanese pattern.

Intake of energy, total, animal and vegetable proteins, Vitamins Bs, C, D, K and electrolytes showed significant increase from T1 to T3 in all three dietary patterns. Alco-

Table 1: General characteristics between the fracture and non fracture groups.

	Fall-related fracture group (n = 28)			Non fall-related fracture group (n = 849)			p value
Age (years old)	82.3	±	5.9	79.1	±	4.6	**0.001**
Height (cm)	151.9	±	9.3	154.6	±	8.7	0.121
Weight (kg)	54.2	±	9.0	57.0	±	9.6	0.121
BMI (kg/m^2)	23.4	±	3.1	23.8	±	3.3	0.528
MMSE	26.6	±	2.7	27.5	±	2.3	0.058
MOS score	3.9	±	1.8	4.3	±	1.7	0.227
Energy intake	2025	±	640	1998	±	495	0.782
Gender (male)		28.6%			44.8%		0.096
Stroke history		7.1%			5.3%		0.671
Diabetes history		17.9%			13.8%		0.541
Kidney disease history		0.0%			7.3%		0.997
Osteoporosis history		3.6%			7.3%		0.462
Cancer history		21.4%			12.8%		0.192
Use of stabilizer		10.7%			13.3%		0.691
Use of hypnotic		7.1%			10.7%		0.549
Use of steroid		3.6%			4.0%		0.908
Use of supplement		10.7%			18.1%		0.589
Use of HRT		39.3%			3.7%		0.998
Smoking habit		21.4%			43.7%		**0.026**
Drinking habit		35.7%			52.1%		0.085
Experience of falls in previous 6 months		25.0%			16.5%		0.252

Variable are presented as mean ± SD or %.
BMI; body mass index, MMSE; Mini Mental State Examination, MOS score; medical outcome study questionnaire, HRT; Hormone Replacement Therapy.
Analysis by Simple logistic regression model
Data in bold are $p < 0.05$

hol intake also significantly increased from T1 to T3 in the Meat pattern and the Traditional Japanese pattern (Additional file 2, Table S4).

Hazard ratio of fall related fractures

The hazard ratios (HR) of fall-related fractures in each dietary pattern are shown in Table 3. The vegetable pattern showed a significant trend for the risk of fall-related fracture. In this pattern, the HR of T3 (confirmed group) compared to T1 (unconfirmed group) was 2.67 (95% CI 1.03 - 6.90) when data was adjusted for age, gender and BMI. The p trend in the Meat pattern for fall-related fracture risk was 0.056 when age, gender, BMI and energy intake were adjusted. The HR of T2 versus T1 in the Meat pattern was 0.36 (95% CI 0.13 - 0.94). Figure 2 indicates the accumulated rate of fall-related fracture onset in tertiles of the Vegetable pattern. The cumulative fall-related fracture incidence in T3 (confirmed) of the Vegetable pattern was higher than T1 or T2. Figure 3 shows that the cumulative fracture incidence in T1 (unconfirmed) of the Meat pattern is higher than T2 or T3. Finally, there was no significant tendency towards fall-related fracture risk in the Traditional Japanese pattern.

Among the 75 food items, vegetables with light green leaves such as lettuce and cabbage (HR = 0.97 for 1 g intake, 95% CI 0.94 - 1.00, p = 0.023) were found to significantly reduce the risk of fall-related fracture when adjusted for age, gender, BMI and energy intake (Table 4). In contrast, seaweed (HR = 1.04, 95% CI 1.00 - 1.08), root vegetables (HR = 1.02, 95% CI 1.00 - 1.03), ice cream (HR = 1.01, 95% CI 1.00 - 1.01) and snacks (HR = 1.04, 95% CI 1.01 - 1.07, p = 0.008) significantly increased the risk of fall-related fracture. The significance of seaweed and roots vegetables was removed when data was adjusted for energy intake. No other food, including dairy products, shellfish, fish, fruit, soybeans and meat showed any rela-

Table 2: Factor analysis for patterns identified (Factor-loading matrix).

	Factor 1: The Vegetable pattern	Factor 2: The Meat pattern	Factor 3: The Traditional Japanese pattern
Radish, Turnip	**0.72**	0.12	0.08
Carrot, Pumpkin	**0.71**	0.01	0.00
Vegetables with light green leaves	**0.64**	0.25	-0.10
Salt intake	**0.59**	0.16	0.11
Vegetable with green leaves	**0.56**	0.19	-0.06
Seaweed	**0.53**	0.15	0.04
Potato	**0.52**	0.00	0.22
Mushroom	**0.51**	0.17	-0.12
Soy product	**0.51**	0.03	0.34
Tomato	**0.49**	0.01	-0.03
Fish	**0.36**	0.26	0.05
Egg	**0.21**	0.20	0.13
Pork, beef, ham, liver	0.08	**0.68**	0.09
Chicken	0.04	**0.61**	0.19
Shellfish, Cuttlefish, Octopus, Shrimp	0.13	**0.53**	-0.05
Noodle	0.22	**0.43**	-0.12
Coffee	0.11	**0.33**	-0.01
Coke	-0.09	**0.31**	0.12
Milk	0.22	**-0.25**	0.15
Pickled vegetable	0.22	**0.24**	0.09
Black tea, Oolong tea	0.08	**0.14**	-0.08
Miso soup	0.17	0.07	**0.72**
Rice	-0.04	0.12	**0.69**
Natto (fermented soybeans)	0.37	-0.05	**0.43**
Persimmon, Strawberry, Kiwi	0.29	-0.01	**-0.41**
Citrus	0.35	-0.07	**-0.39**
Green tea	0.13	-0.03	**0.31**
Percentage of variance (%)	15.5%	7.3%	7.2%

Data for 877 subjects from the FFQ
Data highlighted in bold

tion to the risk of fall-related fracture (p values were over 0.05).

Discussion

The present study is a population based prospective study investigating the relationship between dietary patterns and fall-related fractures in elderly Japanese. Three dietary patterns that appeared in our study are similar to the study of Shimazu et al. who studied Japanese middle age to old age (from 40 to 79) [25]. The Vegetable pattern showed a significant trend for the risk of fall-related fracture. The T3 (confirmed group) showed a significant increase in fall-related fracture risk compared to T1 (unconfirmed group) in the Vegetable pattern. In analysis of each food item, vegetables with light green leaves reduced the fall related fracture risk whereas root vegetables and seaweeds increased the risk. Therefore, not all vegetables increases the risk of fall-related fracture, though the Vegetable pattern showed a significant risk increase overall.

In contrast, T2 (moderately confirmed group) in the Meat pattern showed a significant decrease in fall-related

Table 3: Hazard ratio (95%CI) of fall-related fracture in each dietary pattern.

(3403.8 year*person)

	T1 (unconfirmed)	T2 (moderately confirmed)	T3 (confirmed)	*p* for trend
The Vegetable pattern				
Model 1 Hazard Ratio	1.00 (Reference)	1.13 (0.38-3.36)	**2.67 (1.03-6.90)**	**0.025**
Model 2 Hazard Ratio	1.00 (Reference)	1.11 (0.37-3.31)	**2.66 (1.03-6.87)**	**0.025**
Model 3 Hazard Ratio	1.00 (Reference)	1.12 (0.37-3.39)	2.64 (0.93-7.47)	**0.044**
Model 4 Hazard Ratio	1.00 (Reference)	1.10 (0.36-3.34)	2.62 (0.93-7.41)	**0.044**
The Meat pattern				
Model 1 Hazard Ratio	1.00 (Reference)	0.43 (0.17-1.10)	0.58 (0.23-1.47)	0.211
Model 2 Hazard Ratio	1.00 (Reference)	0.43 (0.17-1.12)	0.58 (0.23-1.47)	0.212
Model 3 Hazard Ratio	1.00 (Reference)	**0.36 (0.13-0.94)**	0.36 (0.12-1.06)	0.056
Model 4 Hazard Ratio	1.00 (Reference)	**0.36 (0.14-0.96)**	0.36 (0.12-1.06)	0.057
The Traditional Japanese pattern				
Model 1 Hazard Ratio	1.00 (Reference)	0.79 (0.33-1.91)	0.80 (0.28-2.28)	0.646
Model 2 Hazard Ratio	1.00 (Reference)	0.81 (0.33-1.96)	0.81 (0.29-2.30)	0.661
Model 3 Hazard Ratio	1.00 (Reference)	0.75 (0.31-1.81)	0.75 (0.26-2.17)	0.561
Model 4 Hazard Ratio	1.00 (Reference)	0.77 (0.32-1.86)	0.76 (0.26-2.19)	0.579

Analysis by Cox proportional hazards model
Model 1: Adjusted by age, gender and BMI
Model 2: Adjusted by Model 1 variable and experience of falls in previous 6 month
Model 3: Adjusted by age, gender, BMI and Energy intake
Model 4: Adjusted by Model 3 variable and experience of falls in previous 6 month
Data in bold are **$p < 0.05$**

fracture risk compared to T1 (unconfirmed group). The trend shown in the meat pattern can be interpreted as T1 group has a tendency to increased risk of fall-related fracture relative to T2 or T3 (see Figure 3).

Our results in dietary pattern analysis appear to contradict previous reports investigating the relationship between dietary patterns and BMD. Tucker *et al.* [13] reported that a dietary pattern with a high consumption of fruit, vegetables and cereals resulted in greater BMD, while Okubo *et al.* [14] showed that a Western pattern with a high intake of fat, meat, butter and seasonings was negatively associated with BMD. Single food item analysis in our study also showed that the variety of vegetables reduces the risk of fall related fracture. Only Xu *et al.* [26] reported that a high intake of meat at a young age reduced the risk of forearm fracture in postmenopausal women. No other researcher has indicated a relationship between the intake of meat and bone health.

Discrepancies between the present and previous studies may be partially explained by differences in population characteristics. All participants in our study were Japanese older than 70 years. The mean meat intake in Japan was only 77.5 g/day in 2002 [27] whereas it reached 242 g/day in the USA in 2000 [28]. Our results should be interpreted as data from a population with low meat consumption. Some nutrients such as proteins and Vitamin Bs contained in meats are known as protective factors for fracture. Figure 2 suggests that the cumulative fall-related fracture incidence in T3 (confirmed) of the Vegetable pattern was higher than T1 or T2. In addition, Figure 3 suggests that the cumulative fracture incidence in T1 (unconfirmed) of the Meat pattern is higher than T2 or T3. In other words, excessive reliance on low meat or high vegetable intake may cause nutritional imbalances and increase the fracture risk in such a population. Nutritional analysis showed an increasing intake of energy, proteins, Vitamin Bs, C, D, K, electrolytes, folic acid and salt from T1 (unconfirmed) to T3 (confirmed) in any dietary pattern. Therefore, all these nutritional factors, not particular ones, may synergistically contribute to the difference of fracture risk among the three dietary patterns.

Dietary patterns should be interpreted in light of regional background. Japanese food culture has been

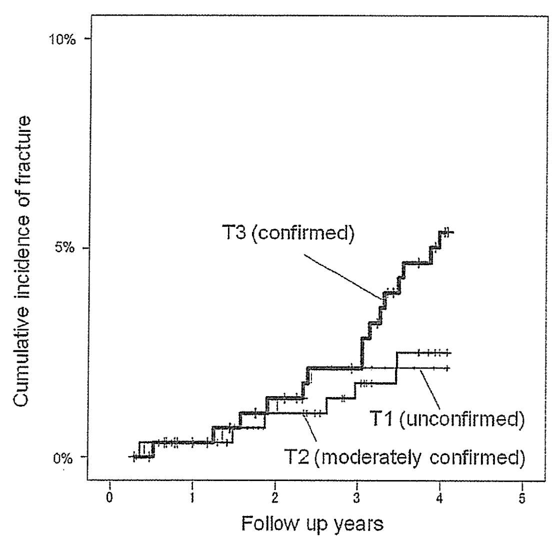

Figure 2 The accumulated rate of fall-related fracture onset in each tertile of the Vegetable pattern. The cumulative fall-related fracture incidence in T3 (confirmed) group of the Vegetable pattern is visibly higher than T1 or T2.

affected by surrounding Asian countries over many years. Interestingly, the greatest naturalist in Chinese history Li Zizhen (1518 to 1593 AD), reported that animal meat such as beef, ram and quail would strengthen bone and muscles in his famous textbook "the General Catalogue of Herbs [29]". Moreover, he stated that light green leaves such as lettuce and cabbage were beneficial for bone health. Our results in a population-based prospective investigation using multivariate analysis may agree more with Li Zizhen than other recent studies.

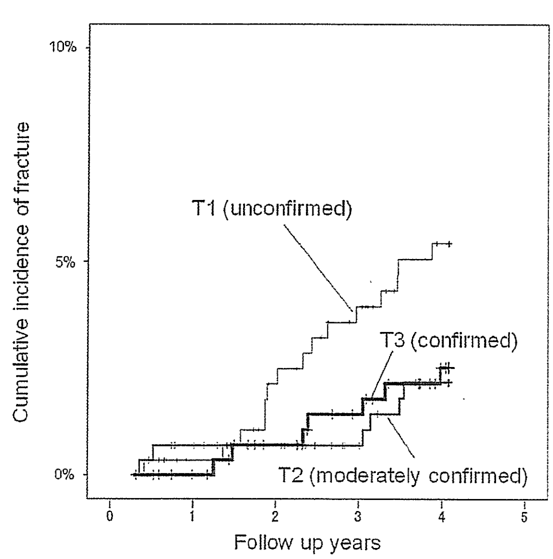

Figure 3 The accumulated rate of fall-related fracture onset in each tertile of the Meat pattern. The cumulative fall-related fracture incidence in T1 (unconfirmed) group of the Meat pattern is higher than T2 or T3.

Our study has several limitations. The number of participants included in the statistical analysis was 877, and the number of fall-related fractures was only 28. Therefore, we were able to adjust few factors in our analysis though many more factors are known to influence the risk of fracture. Also, the limited sample size may affect the statistical detection power. Secondly, though the study design was prospective, dietary data depended on a single cross-sectional investigation in 2002. At that time, all the participants were 70 years old or more, and they were followed for only four years. Therefore, the present study does not reflect long-term dietary habits. Diet at a young age may more strongly influence bone health [26,30]. However, it is very difficult to avoid biases in longitudinal investigations of lifestyle including diet over decades. The FFQ we used was validated against a 3-day diet record in women, designed to give an accurate description of short-term intake information rather than long-term dietary habits.

Though prior falls were known to lead to increasing risk of subsequent falls [31,32], we did not record the number of these falls. The Hazard Ratio of fracture, however, showed no difference after adjustment for experience of falls in the previous 6 months. Though our study was conducted on a particular cohort with certain characteristics (people living in a specific region with official health-care insurance), almost all subjects in Japan use the official medical insurance service we consulted. Therefore, our population is representative of the elderly throughout Japan. This is different to other countries i.e. USA. Finally, age at menopause is known to influence BMD [33] but it was not investigated in the present study.

Despite these limitations, the present study suggests that the impact of dietary patterns in the elderly should not be neglected when assessing the risk of fracture. In a population with low meat consumption, such as elderly Japanese, the moderate consumption of meats may reduce the risk of fall-related fracture. In discussions of diet and health, regional dietary habits should be taken into account.

Conclusion

Dietary patterns were related to the risk of fracture in elderly Japanese. The Vegetable pattern increased the risk of fracture. The Meat pattern had a tendency to reduce the risk of fall-related fracture. These results should be interpreted in light of overall low meat consumption in Japan.

Table 4: Hazard ratio(95%CI) of fall related fracture for each food item (g/day).

			(3403.8 year*person)
	Hazard ratio (95%CI)		p value
Seaweed			
Model 1 Hazard Ratio	**1.04**	**(1.00 - 1.08)**	**0.031**
Model 2 Hazard Ratio	**1.04**	**(1.00 - 1.08)**	**0.032**
Model 3 Hazard Ratio	1.04	(1.00 - 1.08)	0.054
Model 4 Hazard Ratio	1.04	(1.00 - 1.08)	0.056
Root vegetables			
Model 1 Hazard Ratio	**1.02**	**(1.00 - 1.03)**	**0.043**
Model 2 Hazard Ratio	**1.02**	**(1.00 - 1.03)**	**0.041**
Model 3 Hazard Ratio	1.01	(1.00 - 1.03)	0.072
Model 4 Hazard Ratio	1.01	(1.00 - 1.03)	0.069
Snacks, Rice cake, Okonomiyaki			
Model 1 Hazard Ratio	**1.04**	**(1.01 - 1.07)**	**0.008**
Model 2 Hazard Ratio	**1.04**	**(1.01 - 1.07)**	**0.008**
Model 3 Hazard Ratio	**1.04**	**(1.01 - 1.07)**	**0.014**
Model 4 Hazard Ratio	**1.04**	**(1.01 - 1.07)**	**0.016**
Ice cream			
Model 1 Hazard Ratio	**1.01**	**(1.00 - 1.01)**	**0.003**
Model 2 Hazard Ratio	**1.01**	**(1.00 - 1.01)**	**0.004**
Model 3 Hazard Ratio	**1.01**	**(1.00 - 1.01)**	**0.006**
Model 4 Hazard Ratio	**1.01**	**(1.00 - 1.01)**	**0.008**
Vegetables with light green leaves			
Model 1 Hazard Ratio	**0.97**	**(0.94 - 1.00)**	**0.045**
Model 2 Hazard Ratio	**0.97**	**(0.94 - 1.00)**	**0.047**
Model 3 Hazard Ratio	**0.97**	**(0.94 - 1.00)**	**0.023**
Model 4 Hazard Ratio	**0.97**	**(0.94 - 1.00)**	**0.025**

Analysis by Cox proportional hazards model
Model 1: Adjusted by age, gender and BMI
Model 2: Adjusted by Model 1 variable and experience of falls in previous 6 month
Model 3: Adjusted by age, gender, BMI and Energy intake
Model 4: Adjusted by Model 3 variable and experience of falls in previous 6 month
Data in bold are **$p < 0.05$**
Non significant foods were excluded from the table for simplicity.

Additional material

Additional file 1 Table S3: Characteristics of subjects in each tertile of identified dietary patterns.

Additional file 2 Table S4: Nutrition intake of subjects in each tertile of identified dietary patterns.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
YM, KN, KI and NT were responsible for analysis and interpretation of data, and preparation of the manuscript. The first two authors, YM and KN contributed equally to the study. KN and KI were also responsible for the study concept and design. NT carried out the statistical analysis. SK, NY, HA, RN and IT were responsible for the study design. NN and AH are clinical investigators and they contributed to the data analysis. ST, TS and TT contributed to the preparation of the manuscript. All authors read and approved the final version of the manuscript.

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CHEST Original Research
COUGH

Gender Differences in Perceptions of Urge To Cough and Dyspnea Induced by Citric Acid in Healthy Never Smokers

Peijun Gui, BS; Satoru Ebihara, MD, PhD; Masashi Kanezaki, BS; Chihiro Suda, MS; Etsuhiro Nikkuni, BS; Takae Ebihara, MD, PhD; Miyako Yamasaki, MD, PhD; and Masahiro Kohzuki, MD, PhD

Background: **The mechanism of the gender difference in cough reflex threshold has not been clearly elucidated. In the present study, we evaluated gender differences in the cough reflex threshold along with the perceptions of respiratory sensations, urge to cough, and dyspnea.**
Methods: **Nineteen male and 20 female healthy never smokers were recruited through public postings. The cough reflex threshold and the urge to cough were evaluated by inhalation of citric acid. The perception of dyspnea was evaluated by Borg scores during applications of external inspiratory resistive loads.**
Results: **The cough reflex threshold and suprathreshold to citric acid in women, as expressed by the log transformation of the lowest concentration of citric acid that elicited two or more and five or more coughs, was significantly lower than that in men. The urge-to-cough log-log slope in women (1.47 ± 0.81 point × L/g) was significantly steeper than in men (0.96 ± 0.28 point × L/g; $P < .03$). There were no significant differences in the urge-to-cough threshold estimated between men and women. The slope of the dyspnea Borg score change during the external inspiratory resistive loads is steeper in women (0.17 ± 0.04 point/cm H_2O/L/s) than that in men (0.13 ± 0.05 point/cm H_2O/L/s; $P < .01$). The urge-to-cough slope significantly correlated with the perception of dyspnea slope ($r = 0.537$; $P < .01$).**
Conclusions: **The gender difference in cough reflex threshold accompanied the gender difference in amplification rate of respiratory sensations in the same direction. The higher central gain for common pathways for respiratory sensations may play a role in lower cough reflex threshold in women. Further studies are needed to elucidate this issue.** ***CHEST 2010; 138(5):1166–1172***

Abbreviations: C_2 = lowest concentration of citric acid that elicited two or more coughs; C_5 = lowest concentration of citric acid that elicited five or more coughs; R = linear inspiratory resistance

Gender differences exist in airway behavior and clinical manifestation in respiratory diseases.[1] Among them, cough reflex threshold is one of the most dramatic.[2-5] The gender difference in the cough reflex threshold has important clinical implications. In specialty clinics caring for patients with chronic cough, female patients are consistently overrepresented.[6-8] Moreover, a large international population survey found that reporting of nocturnal and nonproductive cough was related to female sex,[9] and a UK survey suggested that daily coughing was more commonly

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reported by women than by men.[10] Despite the clinical significance and dramatic nature, the underlying mechanism of the gender difference in cough reflex threshold has not been clearly elucidated.

The cough reflex usually is referred to as a reflexive defense mechanism mediated at the brainstem level, where sensory information arising from airway sensory receptors in response to an appropriate stimulus is processed by the medullary respiratory network to produce the motor pattern of cough. However, there is accumulating evidence indicating that human cough is under voluntary control and that higher centers such as the cerebral cortex or subcortical regions play an important role in both initiating and inhibiting reflexive cough.[11,12] Although the cough reflex is subjected to influence originating from cortical or subcortical brain regions,[13] understanding of the nature and function of such influences is still limited.

Cough typically is preceded by an awareness of an irritating stimulus and is perceived as a need to cough, termed the "urge to cough."[14] Urge to cough is a component of the brain motivation system that mediates the cognitive responses of cough stimuli.[15] Cough reflex is severely diminished during general anesthesia or sleep.[16,17] In patients with aspiration pneumonia, both the cough reflex and the perception of urge to cough are significantly impaired.[18] These studies suggest that the initiation of a reflex cough response is facilitated by the perception of the urge to cough.

The urge to cough may be mediated, in part, by the cortex, but the awake human does the perceiving. Like any other sensations, the intensity of urge to cough is generated by combination of peripheral afferent inputs and central gain amplification.[19] In the present study, we analyzed the gender difference in the cognition of urge to cough using citric acid as a tussive stimulus. Furthermore, in order to study a possible relationship with other respiratory sensations, we simultaneously investigated the perception of dyspnea during external inspiratory resistive loads.

Materials and Methods

Subjects

Nineteen male and 20 female healthy never smokers were recruited through public postings in and around the Tohoku University School of Medicine campus (Sendai, Japan) to evaluate cough-related responses to inhaled citric acid and dyspnea sensation during inspiratory resistive loads. All were originally recruited. The mean ± SD age was 29.3 ± 7.9 years (range, 20-42 years). Subjects were without history of pulmonary and airway diseases, recent (within 4 weeks) suggestive symptoms, respiratory tract infection, and seasonal allergies. Subjects did not take any regular medication. The study was approved by the Ethics Committee of Tohoku University School of Medicine, and informed consent was obtained from all subjects.

Cough Reflex Threshold and Urge To Cough

Cough reflex, urge to cough, perception of dyspnea, and spirometry were examined at approximately 2:00 PM for each subject. Simple standard instructions were given to each subject.

Cough reflex threshold to citric acid was evaluated with a tidal breathing nebulized solution delivered by an ultrasonic nebulizer (MU-32; Sharp Co Ltd; Osaka, Japan).[18,20] The nebulizer generated particles with a mean mass median diameter of 5.4 μm at an output of 2.2 mL/min. By tidal breathing, one-half of the particles were expected to deposit in the lung.[21] Citric acid was dissolved in saline, providing a twofold incremental concentration from 0.7 to 360 mg/mL. The duration of each citric acid inhalation was 1 min. In the study, cough was defined as a forced expulsive maneuver, usually against a closed glottis, and was associated with a characteristic sound as well as with previous reports about the gender difference in cough reflex threshold.[2-5,22] Based on cough sound, the number of coughs was counted both audibly and visually by laboratory technicians who were unaware of the clinical details of the subjects and the study purpose. Each subject inhaled a control solution of physiologic saline followed by a progressively increasing concentration of citric acid. Increasing concentrations were inhaled until five or more coughs were elicited, and each nebulizer application was separated by a 2-min interval. The cough reflex threshold and suprathreshold were estimated by the lowest concentration of citric acid that elicited two or more coughs (C_2) and the lowest concentration of citric acid that elicited five or more coughs (C_5) during 1 min, respectively.

Immediately after the completion of each nebulizer application, the subject made an estimate of the urge to cough on the modified Borg scale.[14] The Borg scale ranged from no need to cough (0) to maximum urge to cough (10). The urge-to-cough scale was placed in front of the subjects, and each pointed to the scale number, which was recorded by the experimenter. To assess the intensity of the urge to cough, subjects were asked to ignore other sensations, such as dyspnea, burning, irritation, choking, and smoke in the throat. Subjects were told that their sensation of an urge to cough could increase, decrease, or stay the same during the citric acid challenges and that their use of the modified Borg scale should reflect this.

In each subject, the estimated urge-to-cough scores were plotted against the corresponding citric acid concentration using a log-log transformation. Because it is known that a linear relationship exists between estimated urge-to-cough scores and tussive agent concentration on a log-log scale,[14,20] the slope and intersection were determined by linear regression analysis on a log-log scale. The thresholds of urge-to-cough in each subject were estimated as an intersection with the x-axis (citric acid concentration axis), indicating the dose of the urge-to-cough score equaled one.

Perception of Dyspnea

Dyspnea was induced by introducing an inspiratory resistive load to the external breathing circuit and was assessed by the modified Borg scale.[23,24] In brief, the sensation of dyspnea was measured while the subject breathed through a Hans-Rudolph valve with a linear inspiratory resistance (R) of 0, 10, 20, and 30 cm H_2O/L/s. The loads were presented with increasing magnitudes. Neither ventilation nor breathing pattern was controlled during the test. After breathing for 1 min at each level of resistance, the subject rated the sensation of dyspnea (discomfort of breathing) using a modified Borg scale, a category scale from which the subject selected a number from 0 (no dyspnea) to 10 (maximal dyspnea) to describe the magnitude of the sensation of dyspnea. Practically, at the beginning of the measurement, we asked each subject to rate the sensation of *kokyu-konnan* or "discomfort of breathing" while breathing with resistances. The term

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kokyu-konnan is an exact Japanese translation of dyspnea (*kokyu* means breathing or respiration, and *konnan* means discomfort or difficulty). The term *kokyu-konnan* was not defined any further, but the subjects were instructed to avoid rating nonrespiratory sensations, such as headache or irritation of the pharynx.

Comparisons were performed with the Borg score at each load and the summation of the Borg scores of the four loads (R = 0, 10, 20, and 30 cm $H_2O/L/s$). Because it is known that a linear relationship exists between amount of load and dyspnea Borg scores,[25,26] we also estimated the linear regression slope with least-squares fit when estimated Borg scores were plotted against the corresponding amounts of resistive loads.

Data Analysis

Data are expressed as mean ± SD except where specified otherwise. The Mann-Whitney *U* test was used to compare male and female variables. A $P < .05$ was considered significant.

Results

All 39 subjects completed the experiments without any difficulty or side effects. The characteristics of subjects are summarized in Table 1. Age, spirometric measures expressed as percent predicted, and FEV_1/FVC ratio were not statistically significant between men and women.

Figure 1 shows dose-response relationships between citric acid concentration and number of coughs (Fig 1A) or urge to cough (Fig 1B) in individual subjects. As shown in Figure 2A, the cough reflex threshold to citric acid, as expressed by log C_2, in women (0.70 ± 0.36 g/L) was significantly lower than in men (1.13 ± 0.26 g/L; $P < .01$). Similarly, Figure 2B shows that log C_5 in women (0.95 ± 0.41 g/L) was significantly lower than in men (1.32 ± 0.33 g/L; $P < .03$).

The log-log slope between citric acid concentration and the Borg scores of the urge to cough were estimated for each subject. As shown in Figure 3A, the urge-to-cough log-log slope in women (1.47 ± 0.81 point × L/g) was significantly steeper than in men (0.96 ± 0.28 point × L/g; $P < .03$). The urge thresholds were estimated as an intersection with the x-axis of the linear regression equation of the log-log relationships between citric acid concentration and the Borg scores of the urge to cough. There were no significant differences in the urge-to-cough threshold estimated between men (0.33 ± 0.27 g/L) and women (0.14 ± 0.43 g/L) (Fig 3B), suggesting that gender difference in urge to cough was raised from the difference in the central sensitization process rather than from the peripheral sensory inputs.

Table 2 shows the perception of dyspnea during the external inspiratory resistive loads. There were no significant differences between men and women in the Borg scores at R = 0 and 10 cm $H_2O/L/s$. However, the Borg scores at R = 20 and 30 cm $H_2O/L/s$ were significantly greater in women than in men. Figure 4A shows the summation of Borg scores at R = 0, 10, 20, and 30 cm$H_2O/L/s$ for both genders. The scores for women were significantly greater than those for men. When the slope of the dyspnea Borg score change was estimated as a function of the amount of load by linear regression in each subject, the slope for women (0.17 ± 0.04 point/cm $H_2O/L/s$) was significantly steeper than for men (0.13 ± 0.05 point/cm $H_2O/L/s$; $P < .01$) (Fig 4B).

Figure 5 shows the relationship between the urge-to-cough log-log slope obtained in Figure 3A and the dyspnea slope obtained in Figure 4B. The urge-to-cough log-log slope significantly correlated with the dyspnea slope in both men and women. The correlation became stronger for all data in the plot.

Table 1—*Comparison of Characteristics Between Male and Female Sex*

Characteristic	Male Sex	Female Sex	*P* Value[a]
No.	19	20	
Age, y	29.4 ± 6.0	29.2 ± 9.5	ns
FEV_1, % predicted	104.1 ± 26.6	101.0 ± 13.6	ns
FVC, % predicted	108.6 ± 38.5	110.0 ± 13.2	ns
FEV_1/FVC ratio, %	86.5 ± 6.2	89.1 ± 8.2	ns
PEF, % predicted	97.2 ± 14.0	96.7 ± 16.9	ns

Data are presented as mean ± SD, unless otherwise indicated. ns = not significant; PEF = peak expiratory flow.

[a]*P* values were calculated by the Mann-Whitney *U* test.

Discussion

In this study, we found that a lower cough reflex threshold in women was accompanied by an increased slope for log-log relationship in urge-to-cough intensity as a function of citric acid concentrations, whereas the threshold for urge to cough did not differ between men and women. In each gender and when combined, the slope for urge to cough significantly correlated with the slope for perception of dyspnea as a function of imposed external inspiratory resistive loads.

Our use of citric acid data is consistent with previous studies of healthy volunteers using capsaicin[2-4,27] and tartaric acid[5] as tussive stimuli, showing that cough reflex threshold in women is lower than in men. In spite of the solid evidence for the gender difference, the explanation for the decrease in cough reflex threshold among women is unknown. A lowered cough reflex threshold has not been found in female prepubertal children.[28,29] An additional decrease in cough reflex threshold occurs in healthy postmenopausal women.[2] These observational studies suggest that sex hormones may modulate cough reflex threshold, but the current knowledge of the precise mechanism by which sex hormones modulate cough reflex threshold is scanty. There is extensive

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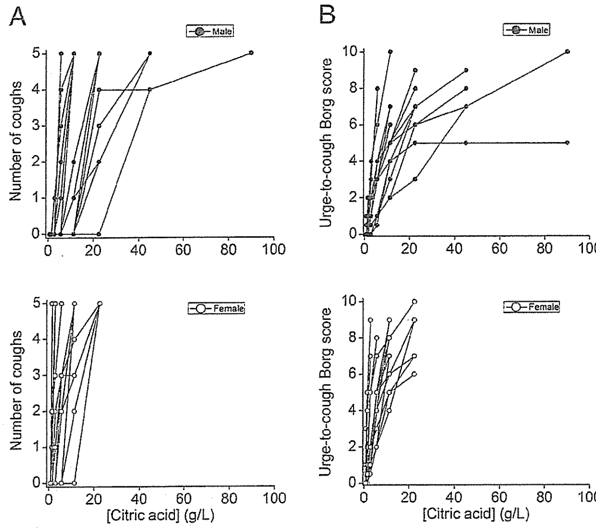

FIGURE 1. Dose-response relationships of citric acid cough challenge test. A, The number of coughs are plotted as a function of citric acid concentration in each subject grouped by sex. B, Borg scores of urge to cough are plotted as a function of citric acid concentration in each subject grouped by sex. In each subject, we stopped the cough challenge test when he or she coughed five times.

literature on the effect of female sex hormones on airway physiology and behaviors in subjects with and without disease,[1,30] but the results are too complex to find relevancy to the gender difference. It is easy to believe that the gender difference of cough reflex threshold might be because of the difference in airway hyperreactivity, but no sex hormone effects in airway responsiveness could be detected.[31] The gender difference in cough reflex threshold may be attributed not only to the peripheral but also to the central effect of sex hormones.

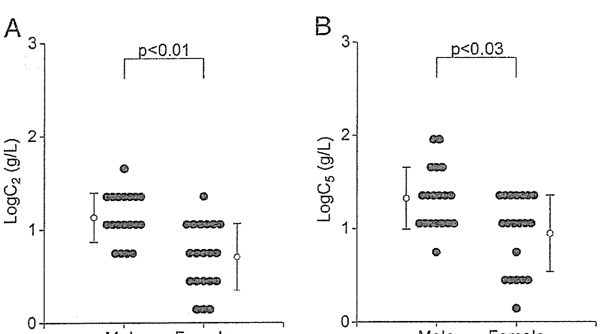

FIGURE 2. Comparisons of cough reflex threshold and suprathreshold between male and female sex. A, Cough-reflex threshold is expressed as the log transformation of the lowest concentration of citric acid that elicited two or more coughs. B, Cough reflex suprathreshold is expressed as the log transformation of the lowest concentration of citric acid that elicited five or more coughs. Open circles and error bars indicate the mean and SD in each group, respectively. C_2 = lowest concentration of citric acid that elicited two or more coughs; C_5 = lowest concentration of citric acid that elicited five or more coughs.

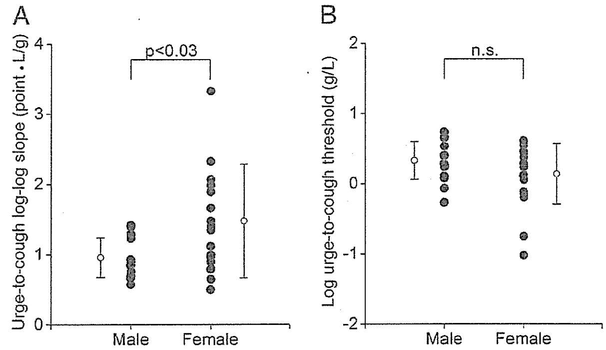

FIGURE 3. Comparisons of urge to cough between male and female sex. A, The urge-to-cough log-log slope by linear regression between log citric acid concentration and the log Borg scores. B, The urge-to-cough threshold estimated by log citric acid concentration at the log Borg score of urge to cough of 0. Closed circles indicate the value for each subject. Open circles and error bars indicate the mean and SD in each group, respectively. n.s. = not significant.

Like any other sensations, the intensity of the urge to cough is generated by a combination of peripheral afferent inputs from peripheral sensors and central gain amplification.[19] It is reasonable to suppose that urge to cough arises from sensors that mediate cough reflex, such as the rapidly adapting receptors, A-δ nociceptors, cough receptors, pulmonary C-fibers, and bronchial C-fibers involved in this reflex.[32] However, there is no report about which sensor causes the urge to cough. The dissociation for cough reflex and urge-to-cough thresholds in this study may suggest that the sensors to induce cough motor action by citric acid and those to induce cough cognitive sensation do not entirely overlap.

There has been extensive recent research on the sensitization of bronchopulmonary sensors related to cough.[33-35] These sensitizations, referred to as a plasticity of sensors, are reflected in intraneuronal mechanisms, such as neuropeptides, in membrane receptors and in transducers.[35] However, in the present study, although cough reflex threshold was lowered and the urge-to-cough log-log slope was heightened in women, the urge-to-cough thresholds did not differ (Fig 3),

Table 2—*Comparison of Perceptions of Dyspnea Between Male and Female Sex*

Perception Score	Male Sex	Female Sex	*P* Value[a]
No.	19	20	
Borg score, points			
R = 0 cm H_2O/L/s	0.3 ± 0.7	0.5 ± 1.0	ns
R = 10 cm H_2O/L/s	1.8 ± 1.2	2.6 ± 1.4	ns
R = 20 cm H_2O/L/s	2.7 ± 1.6	3.8 ± 1.5	< .05
R = 30 cm H_2O/L/s	4.2 ± 1.6	5.6 ± 1.4	< .01

Data are presented as mean ± SD. R = linear inspiratory resistance. See Table 1 legend for expansion of other abbreviation.
[a]*P* values were calculated by the Mann-Whitney *U* test.

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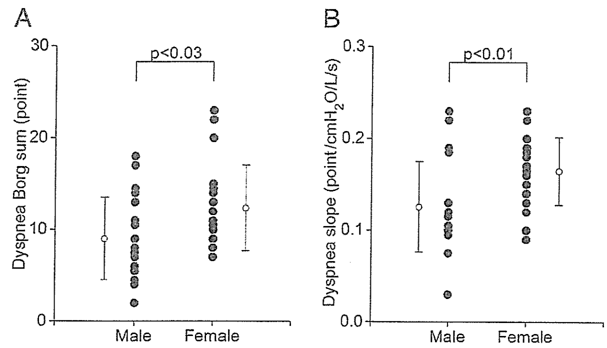

FIGURE 4. Comparisons of perceptions of dyspnea between male and female sex. A, The summation of Borg scores at linear inspiratory resistance of 0, 10, 20, and 30 cm H_2O/L/s. B, Slope calculated from the linear regression slope when estimated dyspnea Borg scores were plotted against the corresponding values of resistive loads. Closed circles indicate the value for each subject. Open circles and error bars indicate the mean and SD in each group, respectively.

suggesting that no significant sensitization in bronchopulmonary sensors are involved in urge-to-cough induction in women.

The urge to cough should not be a passive consequence of the transfer of a defined peripheral input to the sensory cortex but an active process generated partly in the periphery and partly within the CNS by multiple plasticity changes that together determine the gain of the system. It has been proposed that respiratory sensations are the result of neural gating into the cerebral cortex of respiratory afferent inputs[36] and sensory activation of subcortical and cortical neural pathways. Some of these pathways are shared across respiratory modalities (convergent), whereas activation of some neural areas and pathways is modality specific (divergent). Convergent neural mechanisms provide generalized respiratory sensation, such as unpleasantness. In this study, the slopes for urge to cough and dyspnea sensations are steeper in women than in men. Because the two differential slopes are strongly correlated, the gender difference in cough reflex threshold could be based on the common central amplification gain difference for respiratory sensations. Brain imaging studies for dyspnea[37,38] and for the urge to cough[39] suggest that shared brain regions, such as insula, anterior cingulated, and cerebellum, are activated by both dyspnea and urge to cough. If these areas were sensitized in women, the central gain for both urge to cough and dyspnea would be increased.

In addition, the dyspnea sensation induced by external resistive loads is reported to be described as the work-effort sensation of dyspnea.[40-42] The neural pathways proposed for this sensation include corollary discharge from motor cortical centers that drive voluntary breathing and muscle mechanoreceptors and metaboreceptors.[42] Therefore, the peripheral sensors involved in the urge to cough are essentially different from those involved in dyspnea sensations during external resistive loads. Hence, it might be more reasonable to think that the sensitization due to the female sex occurred at the common afferent pathways or somatosensory areas rather than at the peripheral individual sensors.

Regarding the gender difference in perception of dyspnea during external inspiratory resistive loads, although women seem to experience more dyspnea than men, the gender difference was shown to be easily masked by many factors influencing dyspnea.[43,44] In this study, the lowered cough reflex threshold in women is accompanied by both enhancement of perceptions of urge to cough and dyspnea. In an identical protocol, we showed that heightened cough reflex threshold in healthy smokers was accompanied by a decreased cognition of urge to cough, whereas it was not accompanied by the alternation of perception of dyspnea.[45] This difference may suggest a differential site of action between the effects of smoking and gender on the urge to cough.

Heretofore, researchers, including us, conventionally have used C_2 and C_5 to estimate cough-reflex sensitivity, regarding these metrics as threshold and suprathreshold concentrations to sensing tussive stimuli that initiate reflexive circuit activation.[46] However, it is noteworthy that C_2 and C_5 are actually a threshold and a suprathreshold for consequent cough action as a result of neural reflex circuit activation. Our data show that there is no difference between men and women with regard to their sensory thresholds, whereas the slope of the urge-to-cough relationship is enhanced in women, which can explain why

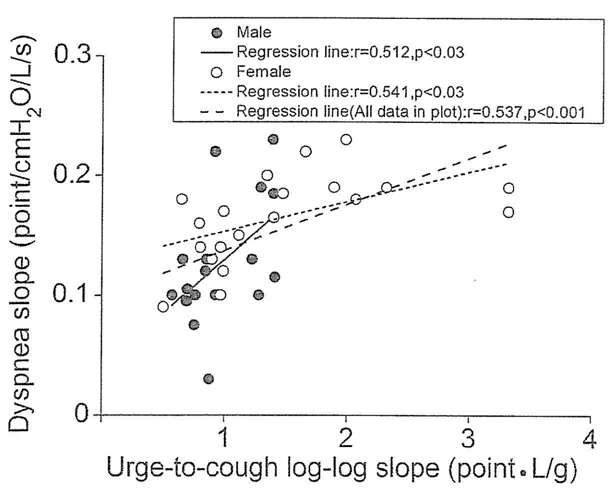

FIGURE 5. Relation between log-log slope in urge to cough and the slope in dyspnea. The regression lines were drawn for male sex (solid), female sex (dotted), and the combination (dashed). Every regression line is significant.

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they cough at a lower citric acid dose than men. Therefore, it may be confusing to use C_2 and C_5 for estimation of cough-reflex sensitivity. Moreover, the dissociation between C_2/C_5 and urge-to-cough thresholds may be attributed to the nature of C_2 and C_5. We need a method to estimate the peripheral sensor sensitivity for cough reflex.

Several reports showed that the urge-to-cough threshold did not differ in spite of differences in the cough reflex threshold or suprathreshold. Nicotine administration significantly raised the C_5 without changing the urge-to-cough threshold.[47] The C_2 and C_5 were greater in smokers than nonsmokers, whereas the urge-to-cough thresholds were not different.[45] However, sample sizes in the reports, including the current study, were small. We may need further studies with larger sample sizes.

We suggest that an enhanced central gain of respiratory sensation exists in women compared with men. Although Kelsall et al[48] clearly showed that the actual cough frequency of female patients is significantly greater than that of male patients with chronic cough, the enhanced respiratory sensations in female patients may contribute to not only the motivation to trigger the reflexive cough but also the motivation to seek medical care, resulting in greater presentation in a cough specialty clinic in women.

It still needs to be clarified how the increased urge-to-cough slope caused the lowered cough reflex threshold in women. The central (postsensor) gain system alternation possibly is involved in this gender difference. Further studies are needed to elucidate the molecular mechanism of gender difference in cough reflex and respiratory sensations.

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Gender Differences in Perceptions of Urge To Cough and Dyspnea Induced by Citric Acid in Healthy Never Smokers

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Cough

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Perception of urge-to-cough and dyspnea in healthy smokers with decreased cough reflex sensitivity

Masashi Kanezaki[1], Satoru Ebihara[1*], Etsuhiro Nikkuni[1], Peijun Gui[1], Chihiro Suda[1], Takae Ebihara[2], Miyako Yamasaki[2], Masahiro Kohzuki[1]

Abstract

Background: Although cigarette smoking has been implicated as an important risk factor for the development of respiratory symptoms, the perceptional aspects of two symptoms in smokers have not been fully elucidated. Therefore, we simultaneously evaluated the cough reflex sensitivity, the cognition of urge-to-cough and perception of dyspnea in both healthy smokers and non-smokers.

Methods: Fourteen male healthy never-smokers and 14 age-matched male healthy current-smokers were recruited via public postings. The cough reflex sensitivity and the urge-to-cough were evaluated by the inhalation of citric acid. The perception of dyspnea was evaluated by Borg scores during applications of external inspiratory resistive loads.

Results: The cough reflex threshold to citric acid, as expressed by the lowest concentration of citric acid that elicited two or more coughs (C_2) and the lowest concentration of citric acid that elicited five or more coughs (C_5) in smokers was significantly higher than in non-smokers. The urge-to-cough log-log slope in smokers was significantly milder than that of non-smokers. There were no significant differences in the urge-to-cough threshold between non-smokers and smokers. There were no significant differences in perceptions of dyspnea between non-smokers and smokers.

Conclusions: The study showed that decreased cough reflex sensitivity in healthy smokers was accompanied by a decreased cognition of urge-to-cough whereas it was not accompanied by the alternation of perception of dyspnea. Physicians should pay attention to the perceptual alterations of cough in smokers.

Background

Cough and dyspnea are common respiratory symptoms for which patients seek medical attention. Although cigarette smoking has been implicated as an important risk factor for the development of respiratory symptoms [1-3], the perceptional aspects of cough and dyspnea in smokers have not been fully elucidated. Since tobacco smoking is also associated with an increase in respiratory and non-respiratory infections [4], it is of importance in a clinical setting to know whether perceptional alternations of these two symptoms occur in smokers, and if so, how they are related. However, there have been few studies which investigated both the perceptions of cough stimuli and dyspneic stimuli in smokers.

Although dyspnea is a respiratory sensation, cough is a motor action typically preceded by a respiratory sensation such as an awareness of an irritating stimulus and is perceived as a need to cough, termed the urge-to-cough [5]. Urge-to-cough is a component of the brain motivation system that mediates the cognitive responses of cough stimuli [6]. Cough reflex sensitivity is severely diminished during general anesthesia or sleep [7,8]. In patients with congenital central hypoventilation syndrome and aspiration pneumonia, both the cough reflex sensitivity and the cognition of cough are significantly impaired [9,10]. These studies suggest that the initiation

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of a cough reflex response is facilitated by the cognition of the urge-to-cough.

Both the urge-to-cough and dyspnea are uncomfortable respiratory sensations. The perceptions of the urge-to-cough and dyspnea may share common pathways and somatosensory areas [11]. Both the urge-to-cough and dyspnea can arise from stimulation by chemical substances and changes in the mechanical environment acting on receptors in the lung and airways [12]. Some pulmonary and airway sensory receptors and afferent pathways may be common to both the urge-to-cough and dyspnea [11]. In addition, brain imaging studies showed the brain cortical areas related to the urge-to-cough and dyspnea overlap [13-15]. Therefore, if the common sensory afferent pathways and/or cortical areas are involved in cough reflex sensitivity which is known to be modulated by tobacco smoking, the perceptions of the urge-to-cough and dyspnea might be changed simultaneously. However, no study has investigated the perception of dyspnea together with cognition of the urge-to-cough in smokers.

Therefore, in the present study, we investigated the cough reflex sensitivity, the cognition of the urge-to-cough and the perception of dyspnea simultaneously in healthy male smokers using citric acid as a tussive stimuli and external inspiratory resistive load as a dyspnea intervention.

Methods

Subjects

Fourteen male healthy never-smokers and 14 male healthy current-smokers were allocated to evaluate cough related responses to inhaled citric acid and dyspnea sensation during inspiratory resistive loads. All were originally recruited via public postings in and around the Tohoku University School of Medicine campus. The mean age was 30.0 ± 4.9 (SD) years. The study was approved by the Institutional Review Board of the Tohoku University School of Medicine. Subjects were without history of pulmonary and airway diseases, recent (within 4 weeks) suggestive symptoms, respiratory tract infection, and seasonal allergies. Subjects did not take any regular medication.

Cough reflex sensitivity and urge-to-cough

Cough reflex, the urge-to-cough, the perception of dyspnea and spirometry were examined at around 2:00 pm for each subject. The smokers smoked more than one cigarette within 2 hours of evaluation. Simple standard instructions were given to each subject.

Cough reflex sensitivity to citric acid was evaluated with a tidal breathing nebulized solution delivered by an ultrasonic nebulizer (MU-32, Sharp Co. Ltd., Osaka, Japan) [10,16]. The nebulizer generated particles with a mean mass median diameter of 5.4 μm at an output of 2.2 ml/min. Citric acid was dissolved in saline, providing a two-fold incremental concentration from 0.7 to 360 mg/ml. The duration of each citric acid inhalation was 1 minute. Based on the "cough sound", the number of coughs was counted both audibly and visually by laboratory technicians who were unaware of the clinical details of the patients and the study purpose. Each subject inhaled a control solution of physiological saline followed by a progressively increasing concentration of citric acid. Increasing concentrations were inhaled until five or more coughs were elicited, and each nebulizer application was separated by a 2 minute interval. The cough reflex sensitivities were estimated by both the lowest concentration of citric acid that elicited two or more coughs (C_2) and the lowest concentration of citric acid that elicited five or more coughs (C_5) during 1 minute.

Immediately after the completion of each nebulizer application, the subject made an estimate of the urge-to-cough. The modified Borg scale was used to allow subjects to estimate the urge-to-cough [5]. The scale ranged from "no need to cough" (rated 0) and "maximum urge-to-cough" (rated 10). The urge-to-cough scale was placed in front of the subjects and the subject pointed at the scale number, which was recorded by the experimenter. To assess the intensity of the urge-to-cough, subjects were recommended to ignore other sensations such as dyspnea, burning, irritation, choking, and smoke in their throat. Subjects were told that their sensation of an urge-to-cough could increase, decrease, or stay the same during the citric acid challenges, and that their use of the modified Borg scale should reflect this.

In each subject, the estimated urge-to-cough scores were plotted against the corresponding citric acid concentration using a log-log transformation. Since it is known that there is a linear relationship between estimated urge-to-cough scores and tussive agent concentration on a log-log scale [5,17], the slope and intersection were determined by linear regression analysis on a log-log scale. The thresholds of the urge-to-cough in each subject were estimated as an intersection with the X-axis (citric acid concentration axis), indicating the dose of the urge-to-cough score = 1.

Perception of dyspnea

Dyspnea was induced by introducing an inspiratory resistive load to the external breathing circuit and was assessed by the modified Borg scale [18,19]. In brief, the sensation of dyspnea was measured while the subject breathed through the Hans-Rudolph valve with a linear inspiratory resistance (R) of 10, 20, and 30 $cmH_2O/L/s$. The loads were presented with increasing magnitudes. Neither ventilation nor breathing pattern was controlled during the test. After breathing for 1 minute at each level of resistance, the subject rated the sensation of dyspnea [discomfort of breathing] using the modified Borg scale. This is a category scale in which the subject selects a number, from 0 (no dyspnea) to 10 (maximal dyspnea), describing the magnitude of the sensation of dyspnea. At the beginning of the measurement each subject was asked to rate the sensation of "kokyu-konnan" or "discomfort of breathing" while breathing with resistances. The term "kokyu-konnan" is an exact Japanese translation of "dyspnea" ("kokyu" means breathing or respiration and "konnan" means discomfort or difficulty). In Japan this is not a special term, and most people understand the meaning of it. The term "kokyu-konnan", or discomfort of breathing was not defined any further, but the subjects were instructed to avoid rating non-respiratory sensations such as headache or irritation of the pharynx.

In order to exclude the mouth piece effect the perception of dyspnea in individuals, the scores at each resistive load were subtracted by the score at R = 0 $cmH_2O/L/s$. After subtractions, comparisons were performed in the Borg score at each load, and summation of the Borg scores of the 3 loads applied. Since it is known that there is a linear relationship between amount of load and Borg dyspnea scores [20,21], we also estimated the linear regression slope with least square fitting when estimated Borg scores were plotted against the corresponding amounts of resistive loads.

Data analysis

The study protocol was approved by the local ethics committee and informed consent was obtained from all subjects. Data are expressed as mean (SD) except where specified otherwise. The Mann-Whitney *U* test was used to compare patients with controls. A p value of < 0.05 was considered significant.

Results

All 28 men completed the experiments without any difficulty or side effects. The characteristics of subjects are summarized in Table 1. There was no significant difference in age, height, body weight, and spirometry data between the non-smokers and smokers. The smokers smoked 12.4 ± 5.7 cigarettes/day for 8.6 ± 4.9 years.

As shown in Figure 1A, the cough reflex threshold to citric acid, as expressed by log C_2, in smokers (1.37 ± 0.36 g/L) was significantly higher than that of non-smokers (0.92 ± 0.39 g/L, p < 0.01). Similarly, the cough reflex threshold to citric acid, as expressed by log C_5, in smokers (1.50 ± 0.35 g/L) was significantly higher than that of non-smokers (1.12 ± 0.43 g/L, p < 0.05) (Figure 1B).

The log-log slope between citric acid concentration and the Borg scores of the urge-to-cough was estimated for each subject. The urge-to-cough log-log slope in smokers (0.83 ± 0.36 points • L/g) was significantly milder than those of non-smokers (1.29 ± 0.47 points • L/g, p < 0.01) (Figure 2A). The urge thresholds were estimated as the intersection with the X-axis (log citric acid concentration) of the linear regression equation of the log-log relationships between citric acid concentration and the Borg scores of the urge-to-cough There were no significant differences in the urge-to-cough threshold estimated between non-smokers (0.22 ± 0.34 g/L) and smokers (0.09 ± 0.49 g/L) (Figure 2B).

Table 1 Comparison of characteristics between non-smokers and smokers

	Non-smokers	Smokers	P- value
Number	14	14	
Age (years)	30.4 ± 3.4	29.6 ± 4.5	n.s.
Height (cm)	173.8 ± 3.5	172.7 ± 4.7	n.s.
Weight (kg)	69.2 ± 13.8	65.9 ± 9.2	n.s.
Pack-years	0 ± 0	5.6 ± 4.9	
FEV_1 (L)	4.16 ± 0.54	4.03 ± 0.46	n.s.
FEV_1 (% predict)	104.5 ± 11.6	101.9 ± 13.0	n.s.
FVC (L)	4.86 ± 0.63	4.64 ± 0.55	n.s.
FVC (% predict)	107.8 ± 30.7	115.2 ± 13.3	n.s.
FEV_1/FVC (%)	85.8 ± 4.6	86.9 ± 3.6	n.s.

Data are mean ± S.D. P-values were calculated by the Mann-Whitney *U* test. n. s. denotes not significant.

Table 2 shows the perception of dyspnea during the external inspiratory resistive loads. There were no significant differences between non-smokers and smokers in the Borg scores at each load and at summation. When the slope of the Borg score change was estimated as a function of the amount of loads by linear regression in each subject, there was no significant difference between non-smokers and smokers

Discussion

In this study, healthy smokers showed a depressed cough reflex sensitivity accompanied by a depressed cognition of the urge-to-cough whereas the perception of dyspnea during external inspiratory resistive loading did not significantly alter.

Both enhanced and diminished cough sensitivities to tussive agents have been reported in chronic smokers [22-26]. The wide range of differences in smoking pattern and history and existing airway dysfunction, were probably related to the balance between up-regulating and down-regulating factors of cough reflex sensitivity. The mechanism of up-regulation of cough reflex sensitivity by tobacco smoking is well characterized in animal studies which consistently show that chronic exposure to cigarette smoke induces enhanced cough responses to various inhaled tussive agents [27-29]. However, the underlying mechanisms for the down-regulation of cough reflex sensitivity in smokers are not fully understood.