Author's response to reviews

Title: Dietary patterns associated with fall related fracture in elderly Japanese: a population based prospective study

Authors:

Yasutake Monma (monmayasutake@mail.tains.tohoku.ac.jp)
Kaijun Niu (ggg@mail.tains.tohoku.ac.jp)
Koh Iwasaki (QFG03604@nifty.com)
Naoki Tomita (n.tomita@ab.cyberhome.ne.jp)
Naoki Nakaya (nakaya-thk@umin.ac.jp)
Atsushi Hozawa (hozawa-thk@umin.ac.jp)
Shinichi Kuriyama (kuriyama-thk@umin.ac.jp)
Takashi Seki (t-seki@m.tains.tohoku.ac.jp)
Takashi Takeda (take@m.tains.tohoku.ac.jp)
Nobuo Yaegashi (yaegashi@mail.tains.tohoku.ac.jp)
Satoru Ebihara (sebihara@idac.tohoku.ac.jp)
Hiroyuki Arai (harai@idac.tohoku.ac.jp)
Ryoichi Nagatomi (nagatomi@m.tains.tohoku.ac.jp)
Ichiro Tsuji (tsuji1@mail.tains.tohoku.ac.jp)

Version: 3 Date: 3 December 2009

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Article title: Dietary patterns associated with fall related fracture in elderly Japanese: a population based prospective study

Yasutake Monma1*, Kaijun Niu2*, Koh Iwasaki18, Naoki Tomita3, Naoki Nakaya4, Atsushi Hozawa4, Shinichi Kuriyama4, Seki Takashi1, Takashi Takeda1, Nobuo Yaegashi1, Satoru Ebihara3, Hiroyuki Arai3, Ryoichi Nagatomi2, and Ichiro Tsuji4

1 Center for Asian Traditional Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan
2 Department of Medicine and Science in Sports and Exercise, Tohoku University Graduate School of Medicine, Sendai, Japan
3 Tohoku University Graduate School of Medicine, and the Department of Geriatrics and Gerontology, Institute of Development, Aging and Cancer, Tohoku University
4 Department of Public Health and Forensic Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

*These authors contributed equally to this work
§Corresponding author

E-mail addresses:
Y M: monmaysutake@m.tains.tohoku.ac.jp
K N: ggg@mail.tains.tohoku.ac.jp
K I: QFG03604@nifty.com
N T: n.tomita@ab.cyberhome.ne.jp
N N: nakaya-thk@umin.ac.jp
A H: hozawa-thk@umin.ac.jp
S K: kuriyama-thk@umin.ac.jp
S T: t-seki@m.tains.tohoku.ac.jp
T T: take@m.tains.tohoku.ac.jp
N Y: yaegashi@mail.tains.tohoku.ac.jp
S E : sebihara@idac.tohoku.ac.jp
H A: harai@idac.tohoku.ac.jp
R N: nagatomi@m.tains.tohoku.ac.jp
I T: tsuji1@mail.tains.tohoku.ac.jp
**ABSTRACT**

**Background:** Diet is considered an important factor for bone health, but is composed of a wide variety of foods containing complex combinations of nutrients. Therefore we investigated the relationship between dietary patterns and fall-related fractures in the elderly.

**Methods:** We designed a population-based prospective survey of 1178 elderly people in Japan in 2002. Dietary intake was assessed with a 75-item food frequency questionnaire (FFQ), from which dietary patterns were created by factor analysis from 27 food groups. The frequency of fall-related fracture was investigated based on the insurance claim record from 2002 until 2006. The relationship between the incidence of fall-related fracture and modifiable factors including dietary patterns were examined. The Cox proportional hazards regression model was used to examine the relationships between dietary patterns and incidence of fall-related fracture with adjustment for age, gender, BMI and energy intake.

**Results:** Among 877 participants who agreed to a 4 year follow-up, 28 suffered from a fall-related fracture. Three dietary patterns, Vegetable pattern, Meat pattern and Traditional Japanese pattern were identified. The moderately confirmed groups in the Meat pattern showed a reduced risk of fall-related fracture (Hazard ratio = 0.36, 95% CI = 0.13 - 0.94) after adjustment for age, gender, Body Mass Index and energy intake. The Vegetable pattern showed a significant risk increase (Hazard ratio = 2.67, 95% CI = 1.03 - 6.90) after adjustment for age, gender and BMI. The Traditional Japanese pattern had no relationship to the risk of fall-related fracture.

**Conclusions:** The moderate confirmation to the meat pattern has the possibility of reducing fall-related fracture risk in elderly Japanese. The results should be interpreted in light of the overall low meat intake of Japanese population.
Background

Fracture accidents of the elderly lead not only reduced activity of daily life [1] but also to increased mortality [2-4]. Diet is considered an important factor for the maintenance of bone health [5-7]. Many nutrients, not only calcium [8,9] and Vitamin D [10], but also phosphorus, vitamin K, strontium, magnesium [11,12], contribute to bone health, but bone is a complex living tissue and a wide spectrum of micronutrients also contribute to its maintenance. Moreover, diets are composed of a wide variety of foods containing complex combinations of nutrients. Therefore, surveys that examine a single nutrient in foods may not adequately account for complicated interactions and cumulative effects on human health.

Tucker et al. [13] and Okubo et al. [14] categorized diets into dietary patterns in order to clarify the relationship between diet and bone mineral density (BMD). Tucker and colleagues reported that a diet with a high fruit and vegetable content appears to have a protective effect on BMD in males while high candy consumption may appear to have a protective effect on BMD in males. Okubo et al. demonstrated 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 persons aged over 70 years living in Tsurugaya. We invited all of these persons to participate in a comprehensive geriatric assessment of medical status, and 1178 participated conveniently and provided written consent and agreed 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 as described below were followed up their incidence of fall related fracture until the end of July 2006. Medical doctor (rehabilitation, exercise medicine, psychiatrist), pharmacist, nurses, and kinesipathists did the baseline assessments.

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 categories were used as unit of measure for statistical analysis. 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 [18].

Assessment of other variables

In addition to diet, we investigated the following factors related to fractures according to WHO report [19]: age, gender, body mass index (BMI) calculated as weight (kg)/height (m) squared, MMSE as a measure of cognitive function, the medical outcome study questionnaire (MOS)[20] 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. All 877 participants including 39 died in the follow-up period were followed until the end of the study, when the physician (R.N.) investigated the clinical records of patients who had fractured. Cases involving traumatic fracture such as traffic accidents were excluded, and only cases involving fall-related fractures were selected as the 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[21]. 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 logistic regression was used to examine the relationships between the risk of a 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[22], BMI[23] 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.

RESULTS
Study population
Of the 877 registered participants, 39 had suffered a fracture by the end of July 2006. Eleven persons had fractures due to traffic accident or other injuries. Therefore, we compared the remaining 28, who fractured due to a fall, to the other 838 participants who had no fractures within our follow up period (Figure 1). 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, experience of smoking or alcohol, and falls in the previous 6 months were compared between the fracture and non fracture group. There were statistically significant differences in age (a median of 80.0 years old in the fracture group and 78.0 years old in the non-fracture group, p=0.001) and Experience smoking (a rate of 22.2% in the fracture group and 44.9% in the non-fracture group, p=0.025) (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, etc.) 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 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 Table 3. Use of steroid and falls in the previous 6 months showed a significant trend in the Vegetable pattern. Weight, BMI and smoking habits showed significance but no trend in the Meat pattern. There was no characteristic showing a significant trend in the Traditional Japanese pattern. On the whole, trends of characteristics within each dietary pattern showed little significance.

Nutrients distributions for T1, T2 and T3 in each dietary pattern are displayed in Table 4. There was no nutrition showing a significant trend in the vegetable and the meat patterns. In the Traditional Japanese pattern, some nutrition including total and animal protein, vitamin B1, C, calcium, magnesium and phosphate showed the significance but no trend.

Hazard ratio of fall related fractures

The hazard ratios (HR) of fall-related fractures in each dietary pattern are shown in Table 5. 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 were 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, rice (HR = 0.54, 95% CI 0.36 - 0.96), dried fish (HR 0.64, 95% CI 0.47 - 0.87) and vegetables with light green leaves such as lettuce and cabbage (HR = 0.74, 95% CI 0.59 - 0.92) were found to significantly reduce the risk of
fall-related fracture when adjusted for age, gender, BMI and energy intake (Table 6). In contrast, seaweed (HR = 1.46, 95% CI 1.01 - 2.10), snacks (HR = 1.37, 95% CI 1.06 - 1.74) and ice cream (HR = 1.37, 95% CI 1.08 - 1.74) significantly increased the risk of fall-related fracture. No other food including dairy products, shellfish, fish, fruit, soybeans and meat showed any relation to the risk of fall-related fracture (p ≥ 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 appeared in our study are close to the previous study of Shimazu T et al. treated Japanese middle age to high age (from 40 to 79)[24]. 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 have a reduced hazard ratio for fall related fracture whereas the roots of 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.

In contrast, T2 (moderately confirmed group) in the Meat pattern showed a significant decrease in fall-related fracture risk compared to T1 (unconfirmed group). The trend shown in the meat pattern can be interpreted that T1 group has a tendency of increased risk of fall-related fracture compared 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 Ohkubo 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 rice, fishes and some kinds of vegetables reduce the risk of fall related fracture. These results are seems to be coincide with previous studies. Only Xu et al. [25] 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. Nutrition analysis in our study showed no significant trend among the three dietary patterns. Therefore, intake of any single nutrition cannot explain the difference of fracture risk among the three dietary patterns. 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 77.5g/day in 2002 [26] whereas it reached 242g/day in the USA in 2000 [27]. Our results should be interpreted as resulting from a study population with low meat consumption. Dietary patterns should be interpreted in consideration of this regional background. Japanese food culture has been affected by surrounding Asian countries over many years. Interestingly, the greatest naturalist in Chinese history Li Zizhen (1518 to 1593 AD), in his famous textbook “the General Catalogue of Herbs [28]”, described that animal meat such as beef, ram and quail would strengthen bone and muscles. Moreover, he noted 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 with Li Zizhen rather than other recent studies.

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, diet 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 [25, 29]. 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. As well, the questionnaire probably gathers less valid intake estimation for men. We did not observe the associated sites of fracture. Prior overall falls increase the risk of
subsequent overall falls[30,31]. But we fail to follow the number of falls. (Though we adjusted the Hazard Ratio of fall related fracture in each dietary pattern, the tendency was the same). Though our study is conducted on a particular sample of persons with certain characteristics (people with health-care insurance in a specific region), almost all subjects in Japan admit the official medical insurance service we used. This is different to other countries i.e. USA. Therefore, our population can be generalized to popular Japanese. Finally, age at menopause is known to influence BMD[32] but this 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 areas with low meat consumption such as Japan, the moderately confirmed group in the meat pattern may reduce the risk of fall-related fracture. In discussions of diet and health, dietary habits of regional residents or of individuals should be taken into account.

**CONCLUSIONS**

Dietary patterns are 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.
Competing interests
None.

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. TT contributed to the preparation of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements
We thank Ms. Kaori Ohmori-Matsuda for her data analysis support.

The present study was supported by a Grant-in-Aid for Scientific Research (13557031); a Grant for Research Conducted by the Japanese Society for Promotion of Science (14010301) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; research grants 2002 and 2003 from the Japanese Atherosclerosis Prevention Fund; and a Health Science Grant on Health Services (H16-seisaku-023) and a Grant for Comprehensive Research on Aging and health (H16-choju-016) from the Ministry of Health, Labor and Welfare of Japan.
REFERENCES


29) Kalkwarf HJ, Khoury JC, Lanphear BP: Milk intake during childhood and


Figure legends

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 as described below were followed up to assess their health condition until the end of July 2006.

Fig 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.

Fig 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) of the Meat pattern is higher than T2 or T3.
Tables and captions

Table 1 – General characteristics between the fracture and non fracture groups
Only age showed a significant difference between the two groups.

Table 2 – Factor analysis for patterns identified (Factor-loading matrix)
Factor 1 was loaded with vegetables, seaweeds, mushrooms, soy products and salt, designated as the Vegetable pattern. Factor 2 was designated as the Meat pattern because it was loaded with meat, processed meat and seafood. Factor 3 was heavily loaded with rice and Miso soup intake. Also, this factor was mildly loaded with Natto. Therefore, we designated this as the Traditional Japanese pattern.

Table 3 – Characteristics of subjects in each tertile of identified dietary patterns
Past falls showed a significant trend in the Vegetable pattern. Smoking habit showed a significant trend in the Meat pattern. On the whole, the trend of characteristics in each dietary pattern showed little significance.

Table 4 – Nutrition intake of subjects in each tertile of identified dietary patterns
There was no nutrition showing a significant trend in the vegetable and the meat patterns. In the Traditional Japanese pattern, some nutrition including total and animal protein, vitamin B1, C, calcium, magnesium and phosphate showed the significance but no trend.

Table 5 – Hazard ratio (95% CI) of fall-related fracture in each dietary pattern
The Vegetable pattern showed a significant trend for the risk of fracture. In this pattern, the HR of T3 compared to T1 was 2.67 (95% CI 1.03 - 6.90) when data was adjusted for age, gender and BMI. On the other hand, the HR of T2 versus T1 in the Meat pattern was 0.36 (95% CI 0.13 - 0.94).

Table 6 – Hazard ratio (95% CI) of fall-related fracture for each food item
In each of the 75 food items, dried fish, vegetables with light green leaves such as lettuce and cabbage and rice significantly reduced the risk of fall-related fracture when adjusted for age, gender, BMI and energy intake. In contrast, seaweed snacks and ice cream significantly increased the risk.