１．発表論文　H 22 年度（2010 年度）

1. Shibata K, Suzuki S, Sato J, Ohsawa I, Goto S, Hashiguchi M and Tokudome S. Abdominal circumference should not be required criterion for the diagnosis of metabolic syndrome. Environ Health Prev Med. 2010; 15: 229-235.

2. Yamada T, Fukatsu M, Suzuki S, Yoshida T, Tokudome S, and Joh T. Alcohol drinking may not be a major risk factor for fatty liver in Japanese undergoing a health checkup. Dig Dis Sci 2010; 55: 176-182.

3. 佐々木敏. 特集：日本人の食事摂取基準（2010年版）策定の考え方. 日本人の食事摂取基準（2010年版）の策定の概要. 静脈経腸栄養 2010; 25: 763-768.

4. Murakami K, Sasaki S, Takahashi Y, Uenishi K; Japan Dietetic Students' Studyfor Nutritio n and Biomarkers Group. Neighborhood socioeconomic status in relation to dietary intake a nd insulin resistance syndrome in female Japanese dietetic students. Nutrition. 2010; 26(5): 508-514.

5. 森田明美. ライフステージ別の栄養 高齢者. 食品保健の科学. 2010; 145-149.

6. Uenishi K, Nakamura K. Intake of dairy products and bone ultrasound measurement in late adolescents: a nationwide cross-sectional study in Japan. Asia Pac J Clin Nutr. 2010; 19(3): 432-439.

7. Uenishi K, Fujita T, Ishida H (他6人). Fractional absorption of active absorbable algal calcium (AAACa) and calcium carbonate measured by a dual stable-isotope method. Nutrients. 2010; 2: 752-761.

8. Tsubota-Utsugi M, Ohkubo T, Kikuya M (他９人). High Fruit Intake is associated with Lower Risk of Future Hypertension determined by Home Blood Pressure Measurement: The OHASAMA study. J Hum hypertens. 2001; Mar;25(3):164-171

9. 野末 みほ, Jun Kyungyul, 石原 洋子, 武田 安子, 永井 成美, 由田 克士, 石田 裕美. 小学5年生の学校給食のある日とない日の食事摂取量と食事区分別の比較. 栄養学雑誌. 2010; 68(5): 298-308.

10. 冨澤 真美, 須藤 美智子, 木村 誠知子, 平野 美知, 石川 俊次, 石田 裕美. 事業所給食施設におけるヘルシーメニューの給与エネルギー量. 栄養学雑誌. 2010; 68(4): 270-275.

11. Kuwabara A, Himeno M, Tsugawa N, Kamao M, Fujii M, Kawai N, Fukuda M, Ogawa Y, Kido S, Okano T, Tanaka K. Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly. Asia Pac J Clin Nutr. 2010; 19(1): 49-56.

12. Kuwabara A, Fujii M, Kawai N, Tozawa K, Kido S, Tanaka K. Bone is more susceptible to vitamin K deficiency than liver in the institutionalized elderly. Asia Pac J Clin Nutr. 2011; 20(1): 50-55.

13. 田中清, 栗原晶子. ビタミン K. 総合臨床. 2010; 59: 594-598.

14. Yoshida M, Hatakeyama E, Hosomi R, Kanda S, Nishiyama T, Fukunaga K. Tissue accumulation and urinary excretion of chromium in rats fed diets containing graded levels of chromium chloride or chromium picolinate. J Toxicol Sci. 2010; 35(4): 485-491.

15. 吉田宗弘. 日本人の微量ミネラルの食事摂取基準（2010）. ビタミン. 2010; 84: 240-245.

２．学会発表　H 22 年度（2010 年度）

上西一弘他、ダブルアイソトープ法による閉経後女性のカルシウム吸収率の検討. 2010 年 10 月 23 日. 第 12 回日本骨粗鬆症学会（大阪）

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REGULAR ARTICLE

Abdominal circumference should not be a required criterion for the diagnosis of metabolic syndrome

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Abstract
Background Metabolic syndrome (MetS) is an established concept. However, it is characterized by a number of different definitions as well as different cut-off points (COPs) for waist circumference (WC) and different modes for incorporating WC into the diagnostic criteria.
Methods Abdominal ultrasonography was performed in 2,333 subjects who also underwent comprehensive medical examinations between April and July 2006. The odds ratios for the number of MetS components were calculated by taking central obesity status into account and considering concurrent fatty liver as an independent variable. We compared the areas under the receiver operating characteristic (ROC) curves for fatty liver and MetS using several MetS criteria.
Results Regardless of the WC criterion selected, we observed a strong linear trend for an association (trend $P < 0.0001$) between MetS and the number of components. The odds ratio (OR) of subjects without central obesity but with all three MetS components was 9.69 (95% confidence interval 3.11–30.2) in men and 55.3 (6.34–483) in women. The COP for the largest area under the curve in men and women was ≥82 cm (OR 0.701) and ≥77 cm (OR 0.699), respectively, when WC was considered as a component. When WC distribution is taken into consideration, practical and appropriate COPs should be ≥85 cm for men and ≥80 cm for women.
Conclusion We suggest that a WC of ≥85 cm for men and ≥80 cm for women would be optimal COPs for the central obesity criteria in the Japanese population. In addition, central obesity should be incorporated as a component of MetS rather than an essential requirement for the diagnosis of MetS.

Keywords Central obesity · Diagnostic criteria · Metabolic syndrome · ROC curve

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Introduction

The prevention of metabolic syndrome (MetS), for which visceral fat accumulation and insulin resistance are considered upstream factors, has recently attracted the attention of the medical world as a useful approach to protect against lifestyle-related diseases typified by arteriosclerotic diseases [1–8]. Visceral fat accumulates for many reasons, including hyperalimentation and inadequate exercise, among others, and causes the abnormal functioning of fat cells and excessive secretion of hormones that are involved in various pathological conditions [9, 10]. Excessive

secretion of these hormones is thought to act in combination with other factors to cause arteriosclerotic and other serious diseases, such as renal failure, blindness, lower limb amputation, cerebral apoplexy, cardiac arrest, and cerebrovascular diseases. The progression of conditions, from obesity into serious diseases, is sometimes referred as the metabolic domino effect [11, 12], and includes fatty liver disease.

Diagnostic criteria for MetS have been published by the World Health Organization [13], American National Cholesterol Education Programs, Adult Treatment Panel III (NCEP–ATP III) [14], and International Diabetes Federation (IDF) [15] for Asian countries, including Japan [16]. In Japan, the Examination Committee for Criteria of MetS introduced diagnostic criteria for Japanese metabolic syndrome (JMetS) [16], which are similar to the ones defined by IDF. The criteria essentially include central obesity and several other components, such as hypertension, hyperglycemia, and abnormal lipid metabolism. In Japan, the most prominent difference between the IDF and Examination Committee criteria for evaluating central obesity is in the cut-off point (COP) for waist circumference (WC), especially that for women: in all countries of the world, with the exception of Japan, the COP for WC is larger for men than that for women.

The relative newness of the MetS concept necessitates that the diagnostic criteria be updated as and when needed. The association between the diagnosis of MetS and downstream diseases in the metabolic domino needs to be addressed in prospective studies. In the study reported here, we applied several criteria to examine the association between metabolic status and concurrent fatty liver, which we used as a specific example of a disease in the metabolic domino. Our aim was to identify preliminary criteria and COPs for WC that can be used in diagnosing MetS.

Subjects and methods

Height, weight, and WC were measured, and abdominal ultrasonography was performed in 2,333 subjects (1,195 men and 1,138 women) of 2,428 subjects aged 40–79 years. These subjects underwent comprehensive medical examinations at the Kasugai City Medical Center during a 3-month period between April and July 2006. Patients receiving drug treatment(s) for liver diseases, hypertension, diabetes mellitus, or hyperlipidemia were excluded from the study. Height and weight were measured using an automatic scale (Tanita BF-220). The WC was measured in standing subjects with a tape measure placed horizontally at the level of the navel while the subject was gently exhaling. If the abdomen was protuberant and the navel was deviated downwards, the tape measure was placed at the midpoint level between the lower intercostal border and the anterior superior iliac spine.

Fatty liver was diagnosed after discussion with medical technologists (including ultrasound technicians), radiology technologists, and physicians and by taking fatty liver scores (as shown in Table 1) obtained at Kasugai City Medical Center into consideration. These scores were based on previous studies [17–20].

Blood pressure was measured on the right arm using a mercury sphygmomanometer; the subject was in a lying position and had rested for at least 5 min prior to the measurement. Venous blood samples were collected in the morning from subjects after a fasting period of 12 h. Triglyceride (TG) and serum high-density lipoprotein cholesterol (HDL-C) were measured by the direct enzymatic method, and fasting plasma glucose (FPG) was measured by the glucose oxidase method. Their concentrations were measured using an automated analyzer (model 7170S; Hitachi, Japan).

Current JMetS criteria require a central obesity (visceral adipose tissue area $\geq$100 cm^2 or WC $\geq$85 cm for men and $\geq$90 cm for women) and two or more of the following three components: (1) high blood pressure, based on a systolic blood pressure $\geq$130 mmHg and/or diastolic blood pressure $\geq$85 mmHg; (2) hyperglycemia, based on FPG $\geq$110 mg/dl; (3) abnormal lipid metabolism, based on TG $\geq$150 mg/dl and/or HDL-C <40 mg/dl [16]. The Examination Committee for Criteria of MetS in Japan also defined a "risk group for MetS" (yobi-gun) consisting of people who have central obesity and one of the three components listed above (high blood pressure, hyperglycemia, or abnormal lipid metabolism). In our study, as in most epidemiological studies, only WC was considered in our evaluation of central obesity; the visceral adipose tissue area was not assessed.

Our primary aim was to identify and propose new MetS criteria based on our results. Our suggested criteria (our criterion 1) considers central obesity not to be an essential requirement for MetS but as only one of the components of MetS. Accordingly, we defined our patients as having MetS when they demonstrated three or more components of

Table 1 Fatty liver score

Condition	Points
Bright echo pattern	0 or 1
Hepatorenal or hepatosplenic contrast	0 or 1 or 2
Unclear vessels	0 or 1
Deep attenuation	0 or 1 or 2
Fatty bandless sign	0 or 1
Liver swelling	0 or 1

A total score of $\geq$3 points is considered to indicate fatty liver

MetS, regardless of their central obesity status. Similarly, the risk group for MetS consisted of those individuals who demonstrated two components.

Taking the number of MetS components listed above in consideration, we first calculated the odds ratios of fatty liver according to central obesity status in men and women by logistic regression. We then constructed receiver operating characteristic (ROC) curves to assess the detecting power of MetS criteria for concurrent fatty liver and calculated the areas under the curve (AUC) for diagnostic criteria. These procedures were repeated using the IDF COP for WC in the Japanese population, i.e., ≥90 cm for men and ≥80 cm for women (our criterion 2). We also calculated the COP for the largest AUC and suggested an optimal COP for men and women based on the study results. Statistical analyses were performed using the SAS system for Windows (release 9.1.3; SAS Institute, Cary, NC), and the AUC value was obtained to refer to the c statistic in PROC LOGISTIC output. All statistical tests were two-sided, and a P value <0.05 was considered to be significant. The study was approved by the ethics committee of Nagoya City University.

Results

Table 2 shows the number of subjects diagnosed with MetS according to the JMetS criteria and our newly proposed criteria, respectively. This diagnosis was based on the number of MetS components, other than central obesity, calculated by WC status in both men and women. Only 8.4% of the women satisfied the central obesity criterion of JMetS, whereas 26.7% men satisfied the criterion. When the COP for central obesity was changed to ≥80 cm, 36.6% of women satisfied the criterion. Among the 13 men and six women who were newly diagnosed with MetS based on our criteria using the same WC COP, seven men (53.8%) and five women (83.3%) had fatty liver. The

Table 2 Criteria of metabolic syndrome and number of subjects

Number of components[a]	Criteria of JMetS	Our criteria	Number of patients diagnosed with MetS	Criteria of JMetS	Our criteria	Number of patients diagnosed with MetS
Men						
	Waist circumference <85 cm			*Waist circumference ≥85 cm*		
0	Normal	Normal	391 (32.7%)	Normal	Normal	93 (7.8%)
1	Normal	Normal	357 (29.9%)	Risk MetS	Risk MetS	152 (12.7%)
2	Normal	Risk MetS	115 (9.6%)	MetS	MetS	61 (5.1%)
3	Normal	MetS	13 (1.1%)	MetS	MetS	13 (1.1%)
Total			876 (73.3%)			319 (26.7%)
	Waist circumference <90 cm			*Waist circumference ≥90 cm*		
0	–	Normal	453 (37.9%)	–	Normal	31 (2.6%)
1	–	Normal	457 (38.2%)	–	Risk MetS	52 (4.4%)
2	–	Risk MetS	151 (12.6%)	–	MetS	25 (2.1%)
3	–	MetS	20 (1.7%)	–	MetS	6 (0.5%)
Total			1,081 (90.5%)			114 (9.5%)
Women						
	Waist circumference <90 cm			*Waist circumference ≥90 cm*		
0	Normal	Normal	603 (53.0%)	Normal	Normal	28 (2.5%)
1	Normal	Normal	357 (31.4%)	Risk MetS	Risk MetS	45 (4.0%)
2	Normal	Risk MetS	76 (6.7%)	MetS	MetS	18 (1.6%)
3	Normal	MetS	6 (0.5%)	MetS	MetS	5 (0.4%)
Total			1,042 (91.6%)			96 (8.4%)
	Waist circumference <80 cm			*Waist circumference ≥80 cm*		
0	–	Normal	458 (40.2%)	–	Normal	173 (15.2%)
1	–	Normal	211 (18.5%)	–	Risk MetS	191 (16.8%)
2	–	Risk MetS	49 (4.3%)	–	MetS	45 (4.0%)
3	–	MetS	4 (0.4%)	–	MetS	7 (0.6%)
Total			722 (63.4%)			416 (36.6%)

JMetS Japanese metabolic syndrome, *Risk MetS* individuals with central obesity and one of three components (high blood pressure, hyperglycemia, or abnormal lipid metabolism), as defined by the Examination Committee for Criteria of MetS in Japan, *MetS* individuals with MetS

[a] Number of the components of MetS other than abdominal obesity

prevalence of fatty liver was much higher than the total prevalence of fatty liver in men and women, i.e., 27.1 and 16.5%, respectively.

Table 3 shows the characteristics of the subjects diagnosed with MetS based on the application of several criteria. The prevalence of MetS using the JMetS criteria was 6.2% in men and 2.0% in women; based on our criteria using the JMetS COP for central obesity, MetS prevalence was 7.3 and 2.5%, respectively. When we applied the criterion for ≥80 cm COP for central obesity in women using our criteria, the prevalence of fatty liver increased to 4.9%. Similarly, the application of the COP increased the prevalence among the MetS risk group to 21.1%, which was close to that observed in men according to our criteria which include the ≥85 cm COP for central obesity. Since central obesity is an essential criterion for determining JMetS or the JMetS risk group, the subjects in these categories are much more obese than those falling in the normal category. The difference in WC and BMI between subjects in the MetS group and the normal group was 12.1 cm and 3.5 kg/m^2, respectively, in men and 17.6 cm and 5.7 kg/m^2 in women. When our criteria were used, these differences decreased to 10.4 cm and 3.0 kg/m^2, respectively, in men and 14.5 cm and 5.0 kg/m^2 in women.

Table 3 Characteristics of the subjects by MetS status

Characteristics	Men			Women		
	Normal	Risk MetS	MetS	Normal	Risk MetS	MetS
Criteria of JMetS (cut-off of WC)	**(85 cm)**			**(90 cm)**		
Number (row%)	969 (81.1%)	152 (12.7%)	74 (6.2%)	1,070 (94.0%)	45 (4.0%)	23 (2.0%)
Fatty liver prevalence (%)	20.6%	46.1%	73.0%	14.5%	40.0%	65.2%
Age (years)	63.0 ± 8.8	63.3 ± 8.4	63.4 ± 7.9	61.6 ± 8.0	65.8 ± 8.1	64.4 ± 6.7
BMI (kg/m^2)	22.3 ± 2.4	25.8 ± 2.4	25.8 ± 2.5	21.7 ± 2.6	27.2 ± 3.4	27.4 ± 3.1
WC (cm)	77.8 ± 6.4	89.6 ± 5.3	89.9 ± 4.9	76.5 ± 7.5	95.0 ± 5.1	94.1 ± 3.7
Systolic blood pressure (mmHg)	122.6 ± 15.1	126.5 ± 16.0	136.2 ± 12.4	122.2 ± 17.0	132.0 ± 13.8	142.3 ± 14.7
Diastolic blood pressure (mmHg)	71.9 ± 8.6	75.0 ± 8.9	79.9 ± 8.2	70.5 ± 9.3	74.6 ± 8.3	79.3 ± 7.0
Triglycerides (mg/dl)	114.3 ± 70.8	142.7 ± 71.6	196.2 ± 150.0	97.5 ± 49.8	120.7 ± 52.7	204.5 ± 101.1
HDL-cholesterol (mg/dl)	62.1 ± 16.4	53.3 ± 12.6	51.9 ± 14.8	72.2 ± 17.1	64.7 ± 14.3	54.1 ± 13.6
Fasting glucose (mg/dl)	96.0 ± 17.2	100.0 ± 18.1	122.9 ± 48.1	92.4 ± 15.8	95.1 ± 13.5	117.3 ± 30.9
Our criteria 1 (cut-off of WC)	**(85 cm)**			**(90 cm)**		
Number (row%)	841 (70.4%)	267 (22.3%)	87 (7.3%)	988 (86.8%)	121 (10.6%)	29 (2.5%)
Fatty liver prevalence (%)	17.6%	43.1%	70.1%	12.7%	35.5%	69.0%
Age (years)	62.7 ± 8.9	63.9 ± 8.1	64.2 ± 8.0	61.3 ± 8.0	64.7 ± 7.9	64.8 ± 6.8
BMI (kg/m^2)	22.2 ± 2.5	24.5 ± 2.7	25.2 ± 2.6	21.7 ± 2.6	24.0 ± 3.7	26.7 ± 3.2
WC (cm)	77.6 ± 6.6	84.9 ± 7.2	88.0 ± 6.7	76.4 ± 7.6	84.3 ± 10.1	90.9 ± 7.6
Systolic blood pressure (mmHg)	120.5 ± 14.1	130.8 ± 15.9	137.1 ± 11.9	120.7 ± 16.3	137.2 ± 14.0	143.9 ± 14.4
Diastolic blood pressure (mmHg)	71.0 ± 8.3	76.1 ± 9.0	79.4 ± 8.0	69.9 ± 9.1	76.8 ± 8.7	79.9 ± 7.5
Triglycerides (mg/dl)	103.8 ± 50.2	159.2 ± 103.1	197.4 ± 140.9	91.2 ± 40.2	152.1 ± 76.4	207.7 ± 92.4
HDL-cholesterol (mg/dl)	63.2 ± 16.0	54.4 ± 15.0	51.3 ± 14.8	73.0 ± 16.8	63.3 ± 16.7	55.2 ± 14.0
Fasting glucose (mg/dl)	93.2 ± 12.2	105.3 ± 24.5	124.5 ± 45.0	91.2 ± 13.9	100.3 ± 21.6	121.9 ± 33.2
Our criteria 2 (cut-off of WC)	**(90 cm)**			**(80 cm)**		
Number (row%)	941 (78.7%)	203 (17.0%)	51 (4.3%)	842 (74.0%)	240 (21.1%)	56 (4.9%)
Fatty liver prevalence (%)	19.6%	50.2%	74.5%	10.3%	27.9%	60.7%
Age (years)	62.8 ± 8.8	64.0 ± 8.2	63.9 ± 7.9	60.6 ± 8.0	64.9 ± 7.2	64.6 ± 7.7
BMI (kg/m^2)	22.5 ± 2.5	24.3 ± 3.0	25.6 ± 3.3	21.3 ± 2.5	23.9 ± 3.1	25.4 ± 2.9
WC (cm)	78.6 ± 6.9	84.3 ± 8.2	88.8 ± 8.6	75.0 ± 7.4	84.3 ± 7.4	87.8 ± 6.6
Systolic blood pressure (mmHg)	121.2 ± 14.4	133.4 ± 15.5	138.2 ± 11.5	118.1 ± 14.9	135.5 ± 15.2	143.1 ± 14.8
Diastolic blood pressure (mmHg)	71.5 ± 8.4	76.9 ± 9.1	80.2 ± 7.9	68.8 ± 8.6	76.1 ± 8.8	79.8 ± 8.0
Triglycerides (mg/dl)	107.6 ± 53.0	172.0 ± 112.7	211.2 ± 172.3	87.0 ± 36.5	126.3 ± 56.4	195.5 ± 102.1
HDL-cholesterol (mg/dl)	62.2 ± 15.9	54.7 ± 16.1	50.1 ± 14.9	74.6 ± 16.6	64.2 ± 15.7	56.4 ± 13.3
Fasting glucose (mg/dl)	93.9 ± 13.4	109.7 ± 28.2	131.1 ± 49.9	90.8 ± 14.4	95.4 ± 14.7	115.5 ± 30.7

Data are given as the mean ± standard deviation (SD)

WC Waist circumference, *BMI* body mass index, *HDL* high-density lipoprotein

When the COP of ≥80 cm was applied, the differences decreased to 12.8 cm and 4.1 kg/m^2, respectively.

Table 4 shows the odds ratios and 95% confidence interval (CI) for fatty liver according to the number of MetS components other than central obesity by WC status. Regardless of sex and the WC COP selected, a strong linear trend was observed for the association (trend $P < 0.0001$) with the number of components. The odds ratio for subjects without central obesity and with all three components of MetS was 9.69 (95% CI 3.1130.2) in men and 55.3 (6.34–483) in women. Using the ≥90 and ≥80 cm COP criterion for central obesity in men and women, respectively, the odds ratio was 55.3 (6.34–483) and 62.4 (6.23–626). These point estimates of odds ratios were higher than those of MetS subjects with two risk factors other than obesity among women, and even among men, they were higher than those of the risk group for MetS who satisfied the central obesity criterion.

Figure 1 shows the ROC curves for the diagnosis of fatty liver according to MetS status by the JMetS criteria and by our criteria. The AUC for the JMetS criteria and our criteria 1 and 2 in men was 0.638, 0.681, and 0.655, respectively. In women, the AUC for our criteria using ≥90 and ≥80 cm COPs for central obesity were 0.625 and 0.681, respectively, whereas that for the JMetS criteria was only 0.570. Based on the findings of our study, the largest AUC was recorded using our criterion 1 (≥85 cm) in men and our criteria 2 in women (≥80 cm). The shapes of the ROC curves of our criterion 2 for men and our criterion 1 for women were very similar, with the coordinates (false positive rate, true positive rate) for MetS and the risk group for MetS being (0.030, 0.188) and (0.204, 0.543), respectively, for men and (0.023, 0.181) and (0.205, 0.537), respectively for women. In addition, when WC was considered as a component, the COP for the largest AUC among men and women was ≥82 cm (0.701) and ≥77 cm (0.699), respectively. We therefore conclude that it would be both practical and appropriate to take WC into consideration, with WC COPs of ≥85 cm for men and ≥80 cm for women. In our study population, 26.7% of the men and 36.6% the women satisfied the criteria.

Table 4 Odds ratio and 95% confidence interval for fatty liver according to the number of the components of MetS other than obesity by waist circumstance status

Number of the components[a]	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval
Men				
	Waist circumference <85 cm		*Waist circumference ≥85 cm*	
0	1.00	Reference	5.49	3.25–9.27
1	1.99	1.32–3.01	7.09	4.51–11.1
2	5.34	3.26–8.74	18.4	9.78–34.4
3	9.69	3.11–30.2	99.7	12.6–786
P for trend	<0.0001		<0.0001	
	Waist circumference <90 cm		*Waist circumference ≥90 cm*	
0	1.00	Reference	7.66	3.59–16.32
1	1.88	1.33–2.66	11.91	6.34–22.39
2	5.17	3.40–7.85	19.96	7.67–51.93
3	14.71	5.45–39.72	31.53	3.62–274.34
P for trend	<0.0001		<0.0001	
Women				
	Waist circumference <90 cm		*Waist circumference ≥90 cm*	
0	1.00	Reference	9.59	4.32–21.3
1	2.32	1.56–3.46	7.37	3.80–14.3
2	5.42	3.10–9.48	17.4	6.45–46.8
3	55.3	6.34–483	44.2	4.85–403
P for trend	<0.0001		<0.0001	
	Waist circumference <80 cm		*Waist circumference ≥80 cm*	
0	1.00	Reference	6.67	3.82–11.7
1	2.67	1.45–4.92	8.63	5.04–14.8
2	6.02	2.70–13.4	26.0	12.5–54.1
3	62.4	6.23–626	125	14.4–1084
P for trend	<0.0001		<0.0001	

[a] Number of the components of metabolic syndrome other than abdominal obesity

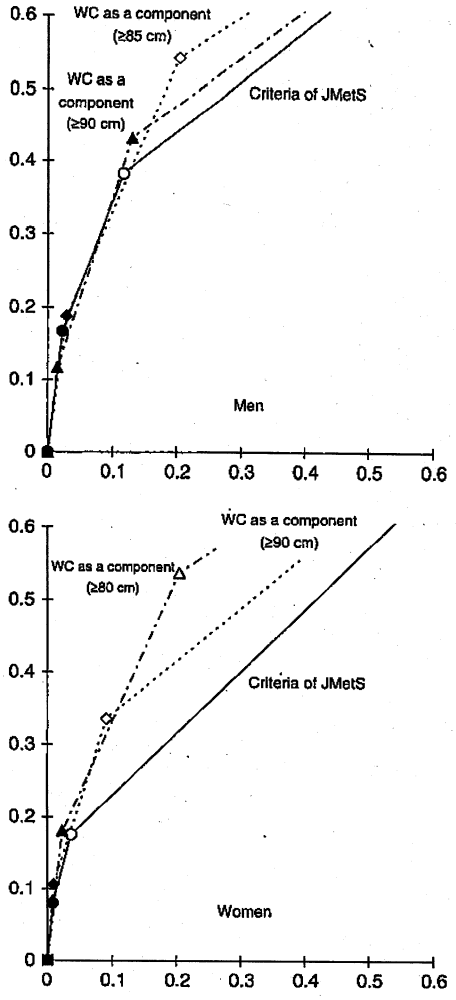

Fig. 1 Receiver operating characteristic curves for fatty liver diagnosis by metabolic syndrome status of several criteria. *JMetS* Japanese metabolic syndrom, *WC* waist circumference

Discussion

In the present study, we considered concurrent fatty liver to be a specific example of a disease in the metabolic domino of MetS and observed that the accumulation of MetS components was associated with higher odds ratios, even without the central obesity component. Taking these results as a whole, we observed stronger associations between MetS and fatty liver in men and women when we considered central obesity as a component rather than an essential requirement for the diagnosis of MetS. We therefore suggest that individuals with an accumulation of components should be regarded as having MetS even in the absence of central obesity, since fatty liver is a component of the metabolic domino. In addition, these individuals may belong to a risk group for other metabolic diseases, including cardiac arrest and cerebrovascular diseases. We also suggest that the optimal COP for WC should be ≥85 cm for men and ≥80 cm for women.

Although the main concepts of MetS are consistent, the COPs for defining central obesity for MetS are controversial, especially in Japan [21]. Several studies have been performed to elucidate the optimal COPs in which ROC analyses with obesity and two or more MetS components other than obesity [22–25] were used. The results suggested that the optimal cut-offs for men and women are 84–90 and 78–82 cm, respectively. Our results are consist with these reported values. However, these earlier studies were based on the internal consistency of obesity and MetS components other than obesity. Further ROC analyses need to be performed to establish the optimal COP for WC, and these should include certain diseases not currently included in MetS. This study is one such analysis.

An important question is whether central obesity should be considered as a requirement for the diagnosis of MetS or as a component of MetS. To answer this question, we need to examine the association between the number of MetS components and particular diseases stratified by central obesity. To date, there have been only two prospective cohort studies [26, 27] from Japan on cardiovascular diseases. Results from NIPPON DATA [26] show the existence of risk accumulation among non-obese subjects, whereas those from Hisayama-cho [27] indicate there is no risk accumulation in such subjects. Data from many studies, including those from our study, are required to facilitate further discussion on this question. However, before the absence of risk accumulation can be established among non-obese individuals, it is possible to treat central obesity as a component of MetS as a precautionary measure.

In general, if a factor is considered to be an essential requirement for the diagnosis of a certain disease, then that factor should not only be etiologically essential but also amenable to accurate measurement in practice; at the very least, the COP should be a sensitive measure. Otherwise, a considerable number of cases would not be detected by the criterion. In fact, the COPs based on the IDF criteria (≥94 cm for men and ≥80 cm for women), with central obesity as a requirement, are more sensitive than those of the NCEP-ATP III criteria (≥102 cm for men and ≥88 cm for women), wherein central obesity is considered a component. Although the JMetS definition is similar to the IDF definition, the JMetS COP for WC in women (≥90 cm) is much less sensitive than the COP of the IDF (≥80 cm). The COP for central obesity for the diagnosis of JMetS is based on the association between visceral fat area and WC [16]. The committee reported that simple correlation analysis of the regression line in women indicated that a WC corresponding to 100 cm^2 of visceral fat was 92.5 cm. However, the correlation coefficient was only 0.65, and more than half of the women with a visceral fat area

$\geq$100 cm^2 would not be found using the WC COP of $\geq$90 cm (meaning that sensitivity is <0.5). The poor sensitivity of the WC in detecting abdominal adiposity is directly linked to the poor sensitivity of the JMetS criteria, in which WC is an essential requirement.

Conclusion

Based on the findings of our study, we suggest that a WC of $\geq$85 cm for men and $\geq$80 cm for women would be optimal COPs for central obesity for the diagnosis of MetS in the Japanese population. We also suggest that central obesity should be used as a component of MetS rather than an essential requirement for the diagnosis of MetS. No definite conclusion has yet been reached regarding the most appropriate diagnostic criteria for MetS. However, within the framework of our study in which fatty liver was considered to be an independent variable, we found that defining abdominal circumference as a component of MetS was less likely to cause errors of oversight and was thus more appropriate than considering abdominal circumference to be a required criterion. The challenge for the future is to identify pathologic conditions that are responsible for MetS and to find better diagnostic criteria through further similar studies that consider factors, other than fatty liver, involved in the metabolic domino effect [11, 12] as independent variables.

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References

1. Matsuzawa Y. Definition and history of metabolic syndrome (in Japanese). Nippon Rinsho. 2006;64:9–12.
2. Wlodarczyk A, Strojek K. Glucose intolerance, insulin resistance and metabolic syndrome in patients with stable angina pectoris. Obesity predicts coronary atherosclerosis and dysglycemia. Pol Arch Med Wewn. 2008;118:719–26.
3. Bulugahapitiya U, Siyambalapitiya S, Sithole J, et al. The clinical impact of identifying metabolic syndrome in patients with diabetes: a cross-sectional study. Diab Vasc Dis Res. 2009;6:21–4.
4. Ford ES, Schulze MB, Pischon T, et al. Metabolic syndrome and risk of incident diabetes: findings from the European prospective investigation into cancer and nutrition-potsdam study. Cardiovasc Diabetol. 2008;7:35.
5. Wannamethee SG, Shaper AG, Lennon L, et al. Metabolic syndrome vs Framingham Risk Score for prediction of coronary heart disease, stroke, and type 2 diabetes mellitus. Arch Intern Med. 2005;165:2644–50.
6. McNeill AM, Rosamond WD, Girman CJ, et al. The metabolic syndrome and 11-year risk of incident cardiovascular disease in the atherosclerosis risk in communities study. Diabetes Care. 2005;28:385–90.
7. Kanauchi M, Kanauchi K, Hashimoto T, et al. Metabolic syndrome and new category 'pre-hypertension' in a Japanese population. Curr Med Res Opin. 2004;20(9):1365–70.
8. Malik S, Wong ND, Franklin SS, et al. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. Circulation. 2004;110:1245–50.
9. Dart AM, Chin-Dusting JP. Lipids and the endothelium. Cardiovasc Res. 1999;43:308–22.
10. Lindsay RS, Wake DJ, Nair S, et al. Subcutaneous adipose 11 beta-hydroxysteroid dehydrogenase type 1 activity and messenger ribonucleic acid levels are associated with adiposity and insulinemia in Pima Indians and Caucasians. J Clin Endocrinol Metab. 2003;88:2738–44.
11. Itoh H. What is 'metabolic domino effect'?—new concept in lifestyle-related disease (in Japanese). Nippon Rinsho. 2003; 61:1837–43.
12. Itoh H. Metabolic domino: new concept in lifestyle medicine. Drugs Today. 2006;42:9–16.
13. World Health Organization. Definition, diagnosis classification of diabetes mellitus, its complications. Part 1: Diagnosis and classification of diabetes mellitus. Geneva: World Health Organization; 1999.
14. Expert panel on detection, evaluation, treatment of high blood cholesterol in adults. Executive summary of the third report of national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. JAMA. 2001;285:2486–97.
15. Alberti KG, Zimmet P, Shaw J. International diabetes federation: a consensus on Type 2 diabetes prevention. Diabetes Med. 2007;24:451–63.
16. The Examination Committee for Criteria of Metabolic Syndrome. Definition and criteria of metabolic syndrome (in Japanese). J Jpn Soc Intern Med. 2005;94:794–809.
17. Taylor KJ, Carpenter DA, Hill CR, et al. Gray scale ultrasound imaging. The anatomy and pathology of the liver. Radiology. 1976;119:415–23.
18. Joseph AE, Dewbury KC, McGuire PG. Ultrasound in the detection of chronic liver disease (the "bright liver"). Br J Radiol. 1979;52:184–8.
19. Kurtz AB, Dubbins PA, Rubin CS, et al. Echogenicity: analysis, significance, and masking. Am J Roentgenol. 1981;137:471–6.
20. Yajima Y, Ohta K, Narui T, et al. Ultrasound in the diagnosis of diffuse liver disease (in Japanese). Rinsho Hoshasen. 1982;27:553–7.
21. Oda E, Abe M, Veeraveedu PT, et al. Considerable disagreement among definitions of metabolic syndrome for Japanese. Circ J. 2007;71:1239–43.
22. Oka R, Kobayashi J, Yagi K, et al. Reassessment of the cutoff values of waist circumference and visceral fat area for identifying Japanese subjects at risk for the metabolic syndrome. Diabetes Res Clin Pract. 2008;79:474–81.
23. Baik I. Optimal cutoff points of waist circumference for the criteria of abdominal obesity: comparison with the criteria of the International Diabetes Federation. Circ J. 2009;73:2068–75.
24. Sato A, Asayama K, Ohkubo T, et al. Optimal cutoff point of waist circumference and use of home blood pressure as a definition of metabolic syndrome: the Ohasama study. Am J Hypertens. 2008;21:514–20.
25. Hara K, Matsushita Y, Horikoshi M, et al. A proposal for the cutoff point of waist circumference for the diagnosis of metabolic syndrome in the Japanese population. Diabetes Care. 2006;29:1123–4.
26. Kadota A, Hozawa A, Okamura T, et al. NIPPON DATA Research Group. Relationship between metabolic risk factor clustering and cardiovascular mortality stratified by high blood glucose and obesity: NIPPON DATA90, 1990–2000. Diabetes Care. 2007;30:1533–8.
27. Doi Y, Ninomiya T, Hata J, et al. Proposed criteria for metabolic syndrome in Japanese based on prospective evidence: the Hisayama study. Stroke. 2009;40:1187–94.

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ORIGINAL ARTICLE

Alcohol Drinking May Not Be a Major Risk Factor for Fatty Liver in Japanese Undergoing a Health Checkup

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Abstract The question of whether alcohol drinking is a risk factor for fatty liver as shown by ultrasonography was investigated by both cross-sectional and longitudinal approaches in Japanese undergoing a health checkup. In this cross-sectional study, 32,438 males (49.0 ± 11.9 years old) and 31,009 females (48.2 ± 11.6 years old) receiving a health checkup from 2000 to 2005 were included. Longitudinally, 5,444 males (49.8 ± 10.7 years old) and 4,980 females (50.4 ± 9.3 years old) participating in both 2000 and 2005 were included. Multiple logistic regression analyses were performed for both sexes, adjusted for age, BMI, and smoking. The prevalence of fatty liver in non-, occasional, daily moderate, and daily heavy drinkers was 28.5, 27.5, 18.7, and 19.1% in men and 12.4, 7.7, 5.4, and 6.7% in women, respectively (inverse association, $P \leq 0.05$ for both). Occasional, daily moderate, and daily heavy drinking in men and occasional and daily moderate drinking in women were inversely associated with fatty liver in the cross-sectional study. Daily moderate and heavy drinking appeared protective in men in the longitudinal study. Alcohol drinking may not be a major risk for fatty liver in Japanese undergoing a health checkup.

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Abbreviations

BMI	Body mass index
OR	Odds ratio
FBG	Fasting blood glucose

Introduction

Fatty liver due to intrahepatic accumulation of lipids is a widely recognized disease, thought to be linked to obesity and alcohol consumption [1–3]. Non-alcoholic fatty liver is recognized as the hepatic consequence of the metabolic syndrome, characterized by abdominal obesity, hypertriglycemia, hyperglycemia, and hypertension [4–6].

It has been controversial whether alcohol drinking causes obesity, although consumption was associated with a greater waist-to-hip ratio, overweight, and fatty liver [7–12]. Alcohol abuse and obesity were found to be equally strong risk factors for fatty liver in the Guangzhou area of China [13]. On the other hand, alcohol drinking may not increase the risk of obesity among US adults, drinking frequency further being inversely associated with the increase in waist circumference and obesity [9–11].

Low to moderate alcohol drinking may lower the risk of type 2 diabetes as well as the metabolic syndrome and cardiovascular mortality [14–19]. Protective effects of low to moderate alcohol drinking on type 2 diabetes may be related to improved insulin sensitivity [20–23]. It is possible that low to moderate alcohol drinking may therefore reduce the fatty liver, which is closely related to insulin resistance [5, 24]. Moderate alcohol drinking may also be a

weaker risk factor for fatty liver than obesity from results for the general population of Northern Italy [25]. Low alcohol drinking, less than 20 g alcohol/day, did not increase the risk for fatty liver in Japanese at a health checkup [26]. Low to moderate alcohol drinking attenuated liver steatosis and non-alcoholic steatohepatitis in severely obese individuals in the USA, possibly by reducing insulin resistance [27]. Moreover, modest wine drinking decreased the prevalence of non-alcoholic fatty liver disease in the Third National Health and Nutrition Survey [28].

Most earlier studies excluded subjects with regular alcohol consumption of more than 20 g/day. Some 54–70% of men and 13% of women in Japan consume more than 23 g alcohol/day [29, 30], drinking behavior being to some extent determined by genetic polymorphisms of alcohol metabolism genes and alcohol-induced liver damage being influenced by the genetic variation of cytochrome P4502EI and alcohol dehydrogenase [31–33]. Therefore, exclusion and selection of categories of drinkers may give rise to misleading results.

In the present cross-sectional and longitudinal investigation, we therefore included all alcohol drinkers in an assessment of risk factors including alcohol drinking for fatty liver assessed by ultrasonography. Adjustment was made for age, body mass index (BMI), and smoking in Japanese undergoing a health checkup.

Methods

Design of Study

This study included both cross-sectional and retrospective longitudinal analyses to investigate whether alcohol consumption, determined by questionnaire, is associated with fatty liver, assessed by ultrasonography, in apparently healthy Japanese undergoing a health checkup. Informed consent was obtained from all participants.

Subjects of the Cross-Sectional Study

A total of 179,646 participants (men: 95,977, 51.7 ± 11.6 years old; women: 83,669, 51.4 ± 11.1 years old) underwent medical examinations including ultrasonography at Okazaki City Medical Association, Public Health Center, between April 2000 and March 2006. Since more than half of the participants repeatedly underwent medical checkups, the participants undergoing a checkup for the first time during this period were included. These comprised 34,593 men and 32,743 women. After exclusion of participants who had past or present histories of hepatic diseases induced by drugs, autoimmune conditions, or unknown etiology based on questionnaire and positive results for hepatitis virus, a total of 63,447 participants (men: 32,438, 49.0 ± 11.9 years old; women: 31,009, 48.2 ± 11.6 years old) were included.

Subjects of the Longitudinal Study

The numbers of participants undergoing medical checkups including ultrasonograpy in 2000 and 2005 were 26,247 (men: 14,627; women: 11,620) and 32,548 (men: 17,207; women: 15,341), respectively. After exclusion of participants who had past or present histories of hepatic diseases induced by drugs, autoimmune conditions, or unknown etiology based on questionnaire and positive results of hepatitis virus, a total of 12,453 participants in both 2000 and 2005 (men: 6,924, 49.5 ± 10.5 years old; women: 5,529, 50.7 ± 9.3 years old) were included. Since 2,029 cases (men: 1,480, 21.4%; women: 549, 9.9%) were assessed as having fatty liver in 2000 on ultrasonography, a total of 10,424 participants (men: 5,444, 49.8 ± 10.7 years old; women: 4,980, 50.4 ± 9.3 years old) without fatty liver in 2000 were longitudinally analyzed to determine risk factors for newly developed fatty liver on ultrasonography in 2005.

Questionnaire

Subjects provided data for alcohol consumption and smoking status in a self-administered questionnaire that was then checked during individual interview by expert nurses in the center. Alcohol consumption was recorded using questions on both frequency and quantity. Frequency of drinking was classified into occasional (1–6 days/week) and daily (7 days/week). One drink was defined as one bottle (500 ml) of beer containing 4–5% alcohol or 1 gou (180 ml) of Japanese sake containing 14% alcohol, which is equivalent to 23 g alcohol [29, 30]. Quantities of drinks were recorded as one, two, or three and more than three drinks per day. Amounts of alcohol consumed per week were estimated by assessing both frequency and numbers of drinks only in the daily drinkers since it was difficult to accurately determine amounts of alcohol in the occasional drinkers. The amounts of alcohol in the participants having daily one, two, and three or more than three drinks were estimated to be 161 g/week, 322 g/week, and 483 g or more than 483 g/week, respectively.

The drinkers were divided into three categories: occasional drinkers, daily moderate drinkers who have one drink (23 g alcohol) per day, and daily heavy drinkers who have two and three or more than three drinks (46 g and 69 g or more than 69 g alcohol, respectively) per day. These categories were determined according to the

previous reports demonstrating that less than 30 g alcohol/day prevented cardiovascular diseases and the risk threshold for alcohol-induced liver disease was more than 30 g alcohol/day [34, 35].

Measurements

Body weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. BMI was categorized into three categories: <25, 25–29.9, and ≤30 according to the criteria determined by the Japan Society for the Study of Obesity. Age was categorized into four categories: <40, 40–49, 50–59, and <60.

Blood samples were taken from each participant after overnight fasting. Fasting blood glucose (FBG) was measured by Hitachi autoanalyzer models 7600 and 7700 (Hitachi Medical, Co., Tokyo, Japan).

Fasting hyperglycemia was defined if serum FBG was ≤110 mg/dl. Elevated blood pressure or hypertension was diagnosed if resting blood pressures was ≤130/85 mmHg or if the participants had either a history of hypertension or antihypertensive medication, respectively.

Abdominal ultrasonographic examination was performed using convex-type real-time electronic scanners (SSA 250 and 300, Toshiba Medical, Co., Tokyo, Japan) by ten technicians lacking any information about the subjects, including alcohol history. All images were printed on sonograph paper and reviewed by other technicians and physicians. Fatty liver was assessed according to the modified criteria reported previously [36, 37]. These include a comparative assessment of liver brightness (diagnosed by a difference of more than 10 in the average liver and renal cortical echo amplitudes), attenuation of echo penetration, and decreased visualization of veins.

Statistical Analyses

Multiple logistic regression analyses were performed to determine the influence of drinking as a risk factor for fatty liver in both men and women, both adjusted for age and for age, BMI, and smoking in the cross-sectional and longitudinal studies. Adjustment was also made for age, BMI, smoking, and either FBG or elevated blood pressure and hypertension. The analyses were further performed after excluding daily heavy drinkers.

Statistical differences among groups were identified using one-way analysis of variance, followed by multiple comparisons using Bonferroni method. The $m \times n$ chi-square test and Fisher's test were used for comparison of prevalence of fatty liver. Logistic regression analyses were performed using computer software (SPSS version 13.0 for Windows). P values less than 0.05 were considered significant.

Results

Cross-Sectional Study

The percentages of occasional, daily moderate, and daily heavy drinkers were 32.9, 17.7, and 9.3% overall, 33.8, 27.6, and 16.5% for men, and 32.1, 7.4 and 1.8% for women, respectively. Age was significantly lower in occasional and daily drinkers than in non-drinkers in both sexes (Table 1). BMI was significantly higher in occasional drinkers and lower in daily drinkers than in non-drinkers in men and was significantly lower in occasional and daily drinkers than in non-drinkers in women. In addition, the overall prevalence of fatty liver was 23.9% in men and 10.3% in women, and the prevalence of fatty liver in daily

Table 1 Age, BMI, prevalence of fatty liver, and ever smoking rates due to drinking habits in the cross-sectional study

	Non-drinkers	Occasional drinkers	Daily moderate drinkers	Daily heavy drinkers
Men				
%	21.7	33.8	27.6	16.5
Age	50.9 ± 12.6	46.4 ± 12.1*	50.7 ± 11.2	49.1 ± 10.7*
BMI	23.1 ± 3.2	23.4 ± 3.1*	22.9 ± 2.8	23.0 ± 2.8
Fatty liver (%)	28.5	27.5	18.7	19.1
Ever smoking rates (%)	41.1	41.3	44.4	59.6
Women				
Number (%)	58.5	32.1	7.4	1.8
Age	50.6 ± 11.4	44.3 ± 11.2*	47.5 ± 10.0*	42.7 ± 10.1*
BMI	22.2 ± 3.3	21.7 ± 3.1*	21.4 ± 2.8*	21.2 ± 3.0*
Fatty liver (%)	12.4	7.7	5.4	6.7
Ever smoking rates (%)	5.9	11.6	17.3	52.4

* $P < 0.05$ compared with non-drinkers

drinkers was significantly lower than in non-drinkers in both sexes.

Multiple logistic regression analysis revealed that occasional and daily moderate drinking both adjusted for age and for age, BMI, and smoking was inversely associated with fatty liver in both sexes (Table 2). Daily heavy drinking fully adjusted for other factors was inversely associated with fatty liver in men, while this relation did not reach statistical significance in women.

Adding FBG or elevated blood pressure and hypertension, the ORs were not changed in both sexes. After removing the daily heavy drinkers (5,370 men and 563 women), the results were not essentially changed (data not shown).

Longitudinal Study

The percentages of occasional, daily moderate, and daily heavy drinkers were 30.6, 20.3, and 9.5% overall, 31.3, 32.3, and 17.0% for men, and 29.9, 7.0, and 1.2% for women, respectively. Age was significantly lower in occasional and daily heavy drinkers in men and in three groups of drinkers in women than in non-drinkers (Table 3). Fatty liver newly developed in 10.2, 12.1, 11.7, and 12.0% of non-, occasional, daily moderate, and daily heavy drinkers, respectively, overall within the 5-year period. Fatty liver was found in 16.4, 16.7, 12.9, and 12.4% of non-, occasional, daily moderate, and daily heavy drinkers in men, respectively, and in 8.2, 6.8, 5.7, and 6.7% of the women, respectively. The risk of newly developed fatty liver was significantly lower in daily moderate and heavy drinkers than non-drinkers in men.

In the multiple logistic regression analysis, daily moderate and heavy drinking was inversely associated with fatty liver adjusted for age, BMI, and smoking in men. Although similar inverse association was observed in women, this did not reach statistical significance (Table 4). Adding FBG or elevated blood pressure and hypertension did not alter the ORs (data not shown). After removing the daily heavy drinkers (928 men and 60 women), daily moderate drinking was the inverse risk factor for fatty liver (ORs 0.72, 95% CI 0.58–0.89) in men, while the results were not changed in women.

Table 2 Multiple logistic regression analysis for fatty liver in the cross-sectional study

	Age-adjusted OR	95% CI	Multivariate OR*	95% CI
Men				
Non-drinkers	1.00	References	1.00	References
Occasional drinkers	0.93	0.87–0.99	0.89	0.83–0.96
Daily moderate drinkers	0.56	0.52–0.60	0.58	0.53–0.63
Daily heavy drinkers	0.56	0.51–0.61	0.57	0.52–0.63
Women				
Non-drinkers	1.00	References	1.00	References
Occasional drinkers	0.74	0.68–0.81	0.77	0.70–0.85
Daily moderate drinkers	0.44	0.37–0.53	0.53	0.43–0.64
Daily heavy drinkers	0.70	0.50–0.98	0.85	0.60–1.23

* Adjusted by age, BMI, and smoking status

Table 3 Age, BMI, and ever smoking rates due to drinking habits in the longitudinal study

	Non-drinkers	Occasional drinkers	Daily moderate drinkers	Daily heavy drinkers
Men				
Number (%)	19.1	31.3	32.3	17.0
Age	51.4 ± 11.2	48.7 ± 11.1*	50.3 ± 10.5	49.0 ± 9.5*
BMI	22.2 ± 2.6	22.5 ± 2.5*	22.4 ± 2.4	22.4 ± 2.4
Ever smoking rates (%)	39.0	41.8	44.6	63.9
Women				
Number (%)	61.5	29.9	7.0	1.2
Age	51.8 ± 9.2	47.9 ± 9.2*	49.6 ± 8.6*	46.8 ± 9.0*
BMI	21.8 ± 2.6	21.8 ± 2.6	21.5 ± 2.5	21.5 ± 2.7
Ever smoking rates (%)	4.3	9.2	17.7	53.5

* $P < 0.05$ compared with non-drinkers

Table 4 Multiple logistic regression analysis for fatty liver in the longitudinal study

	Age-adjusted OR	95% CI	Multivariate OR*	95% CI
Men				
Non-drinkers	1.00	References	1.00	References
Occasional drinkers	0.97	0.78–1.19	0.95	0.77–1.17
Daily moderate drinkers	0.73	0.59–0.90	0.72	0.58–0.89
Daily heavy drinkers	0.67	0.52–0.87	0.65	0.50–0.85
Women				
Non-drinkers	1.00	References	1.00	References
Occasional drinkers	0.83	0.65–1.05	0.81	0.63–1.04
Daily moderate drinkers	0.67	0.42–1.07	0.71	0.44–1.16
Daily heavy drinkers	0.08	0.29–2.26	0.74	0.25–2.17

* Adjusted by age, BMI, and smoking status

Discussion

The present study demonstrated that alcohol drinking may not be a major risk factor for fatty liver as assessed by ultrasonography in Japanese undergoing a health checkup. Thus, the prevalence of fatty liver in both sexes was significantly lower in daily drinkers than in non-drinkers. Occasional, daily moderate, and daily heavy drinking in men and occasional and daily moderate drinking in women fully adjusted for other factors were inversely associated with fatty liver in the cross-sectional study. Daily moderate and heavy drinking exerted protective effects against the development of fatty liver in men in the longitudinal study.

The low to moderate amounts of alcohol found to reduce type 2 diabetes, metabolic syndrome, and cardiovascular diseases have ranged widely [14–23]. However, low to moderate amounts of alcohol were usually defined as less than 30 g alcohol/day [34, 35, 38]. Further, the risk for cardiovascular diseases is lower when alcohol consumption is low to moderate, and the risk is higher when alcohol consumption is high, resulting in a dose-response curve that is J- or U-shaped [38]. It was also demonstrated that the threshold for non-cirrhotic and cirrhotic liver damage was reported to be less than 30 g alcohol/day, and risk increased with increasing daily intake [35, 39]. We estimated that alcohol consumption of daily heavy drinkers ranged from 46 g alcohol/day to 69 g or more than 69 g alcohol/day in the present study. We also demonstrated that even daily heavy drinking was inversely associated with fatty liver and that exclusion of daily heavy drinkers did not essentially alter the trend in both cross-sectional and longitudinal studies. However, we do not encourage heavy alcohol drinking since we focused the effect on fatty liver, but not on liver injury, and more than 30 g alcohol/day has been reported to be injurious to the liver [35, 39].

Ethanol is known to impair fat oxidation and stimulate lipogenesis in the liver [2, 3]. Although there is conflicting evidence, alcohol intake is reported to be associated with fatty liver in apparently healthy adult men in Spain, with alcohol abuse and obesity being equally strong risk factors for fatty liver in the Guangzhou area of China [12, 13]. Alcohol drinking was found to be a weaker risk factor for fatty liver than obesity in another study [25].

Although our results appear paradoxical on the surface, we speculate that the discrepancy may be related to the different proportion of heavy alcohol drinkers. Our results are in line with other reports that low alcohol drinking did not increase the risk for fatty liver in health checkup participants in Japan and that low to moderate alcohol drinking reduced liver steatosis and non-alcoholic steatohepatitis found in the severely obese in the USA [26, 27]. Further, it was recently demonstrated that modest wine consumption was associated with a reduced prevalence of non-alcoholic fatty liver disease [28].

Adding FBG or elevated blood pressure and hypertension did not alter the ORs in both cross-sectional and longitudinal studies, suggesting that the relationship between alcohol drinking and fatty liver was not confounded by these factors and the effect of alcohol drinking on fatty liver may be independent of improved glucose metabolism and endothelial function. The mechanism by which low to moderate alcohol drinking reduces type 2 diabetes, cardiac ischemic diseases, and the metabolic syndrome may be, in part, related to increased insulin sensitivity [20–23]. Insulin resistance causes accumulation of fat in the hepatocytes through lipolysis and hyperinsulinemia [4, 40]. Although we did not measure insulin sensitivity in the present study, we speculate that this may be increased in our population by alcohol drinking, thereby attenuating fatty liver.

A major limitation of the present study was the cross-sectional and retrospective longitudinal design. The subjects were limited to the Japanese participants undergoing a health checkup. Although it would have been preferable to follow up all participants in 2000 to investigate the risk factor for fatty liver in 2005 in a cohort manner, only 42.5% of the participants in 2000 received the medical checkup in 2005. In addition, alcohol consumption was

self-reported, and the drinkers were roughly divided into four groups according to the frequency of drinking for logistic regression analyses, which may result in inaccuracies. Finally, although histological diagnosis is more accurate, we had to rely on ultrasonography for the purposes of the present study. Ultrasonography cannot distinguish steatosis and steatohepatitis, with the result that it may be unclear if the participants drinking alcohol have liver damage. However, it has been widely used to assess fatty liver since it is a non-invasive procedure with relatively high sensitivity and specificity for screening purposes [1, 12, 13, 25, 26, 36, 37]. The prevalence of fatty liver, 23.9% in men and 10.3% in women in the present study, is consistent with values in a previous Japanese report [41].

In conclusion, alcohol drinking may not be a major risk factor for fatty liver on ultrasonography in Japanese undergoing a health checkup. However, we should be prudent, and the available data do not yet provide a rationale for encouragement of alcohol consumption. Future cohort studies assessing the influence of differing amounts of alcohol are necessary to confirm whether alcohol drinking may indeed not be a risk for fatty liver.

References

1. Lin YU, Lo HM, Chen JD. Sonographic fatty liver, overweight and ischemic disease. *World J Gastroenterol*. 2005;11:4838–4842.
2. You M, Crabb DW. Recent advances in alcoholic liver disease II. Minireview: molecular mechanisms of alcoholic fatty liver. *Am J Physiol*. 2004;287:G1–G6. doi:10.1152/ajpcell.00559.2003.
3. You M, Fischer M, Deeg MA, Crabb DW. Ethanol induces fatty acid synthesis pathways by activation of sterol regulatory element-binding protein (SREBP). *J Biol Chem*. 2002;77:29342–29347. doi:10.1074/jbc.M202411200.
4. Angelico F, Del Ben M, Conti R, et al. Non alcoholic fatty liver syndrome: a hepatic consequence of common metabolic diseases. *J Gastroenterol Hepatol*. 2003;18:588–594. doi:10.1046/j.1440-1746.2003.02958.x.
5. Marchesini G, Bugianesi E, Forlani G, et al. Non alcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*. 2003;37:917–923. doi:10.1053/jhep.2003.50161.
6. Akbar DH, Kawther AH. Non alcoholic fatty liver disease and metabolic syndrome: what we know and what we do not know. *Med Sci Monit*. 2006;12:RA23–RA26.
7. Lukasiewics E, Mennen LI, Bertrais S, et al. Alcohol intake in relation to body mass index and waist-to-hip ratio: the importance of type of alcoholic beverage. *Public Health Nutr*. 2005;8:3 15–320.
8. Dallongeville J, Marécaux N, Ducimetiére P, et al. Influence of alcohol consumption and various beverages on waist girth and waist-to-hip ratio in a sample of French men and women. *Int J Obes Relat Metab Disord*. 1998;22:1178–1183. doi:10.1038/sj.ijo.0800648.
9. Liu S, Serdula MK, Williamson DF, Monkdad AH, Byers T. A prospective study of alcohol intake and change in body weight among US adults. *Am J Epidemiol*. 1994;140:912–920.
10. Tolstrup JS, Heitmann BL, Tjønneland AM, Overvad OK, Sørensen TI, Grønbaek MN. The relation between drinking pattern and body mass index and waist and hip circumference. *Int J Obes*. 2005;29:490–497.
11. Tolstrup JS, Halkjaer J, Heitmann BL, et al. Alcohol drinking frequency in relation to subsequent changes in waist circumference. *Am J Clin Nutr*. 2008;87:957–963.
12. Parés A, Tresserras R, Nunez I, et al. Prevalence and factors associated to the prevalence of fatty liver in apparently healthy adult men. *Med Clin (Barc)*. 2000;114:561–565.
13. Chen QK, Chen HY, Huang KH, et al. Clinical features and risk factors of patients with fatty liver in Guangzhou area. *World J Gastroenterol*. 2004;10:899–902.
14. Fuchs CS, Stampfer MJ, Colditz GA, et al. Alcohol consumption and mortality among women. *N Engl J Med*. 1995;332:1245–1250. doi:10.1056/NEJM199505113321901.
15. Freiberg MS, Cabral HJ, Heeren TC, Vasan RS, Curtis Ellison R. Alcohol consumption and the prevalence of the metabolic syndrome in the US: a cross-sectional analysis of data from the Third National Health and Nutrition Examination Survey. *Diabetes Care*. 2004;27:2954–2959. doi:10.2337/diacare.27.12.2954.
16. Dixon JB, Dixon ME, O'Brien PE. Alcohol consumption in the severely obese: relationship with the metabolic syndrome. *Obes Res*. 2002;10:245–252. doi:10.1038/oby.2002.33.
17. Zilkens RR, Burke V, Watts G, Beilin LJ, Puddey IB. The effect of alcohol intake on insulin sensitivity in men: a randomized controlled trial. *Diabetes Care*. 2003;26:608–612. doi:10.2337/diacare.26.3.608.
18. Baer DJ, Judd JT, Clevidence BA, et al. Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. *Am J Clin Nutr*. 2002;75:593–599.
19. Wannamethee SG, Shaper AG, Perry IJ, Alberti KGMM. Alcohol consumption and the incidence of type II diabetes. *J Epidemiol Community Health*. 2002;56:542–548. doi:10.1136/jech.56.7.542.
20. Kiechl S, Willeit J, Poewe W, et al. Insulin sensitivity and regular alcohol consumption: large, prospective, cross sectional population study (Bruneck study). *BMJ*. 1996;313:1040–1044.
21. Meyer KA, Conigrave KM, Chu NF, et al. Alcohol consumption patterns and HbA1c, C-peptide and insulin concentrations in men. *J Am Coll Nutr*. 2003;22:185–194.
22. Wakabayashi I, Kobaba-Wakabayashi R, Masuda H. Relation of drinking alcohol to atherosclerotic risk in type 2 diabetes. *Diabetes Care*. 2002;25:1223–1228. doi:10.2337/diacare.25.7.1223.
23. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. *Diabetes Care*. 2005;28:719–725. doi:10.2337/diacare.28.3.719.
24. Bugianesi E, McCullough AJ, Marchesini G. Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology*. 2005;42:987–1000. doi:10.1002/hep.20920.
25. Bellentani S, Saccoccio G, Masutti F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med*. 2000;132:112–117.
26. Hamaguchi M, Kojima T, Takeda N, et al. The metabolic syndrome as a predictor of non alcoholic fatty liver disease. *Ann Intern Med*. 2005;143:722–728.
27. Dixon JB, Bhathal PS, O'Brien PE. Non alcoholic fatty liver disease: predictors of non alcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology*. 2001;121:91–100. doi:10.1053/gast.2001.25540.
28. Dunn W, Xu R, Schwimmer JB. Modest wine drinking and decreased prevalence of non alcoholic fatty liver disease. *Hepatology*. 2008;47:1947–1954. doi:10.1002/hep.22292.

29. Kawado M, Suzuki S, Hashimoto S, et al. Smoking and drinking habits 5 years after baseline in the JACC study. *J Epidemiol.* 2005;15:S56–S66. doi:10.2188/jea.15.S56.
30. Sakata K, Hoshiyama Y, Morioka S, et al. Smoking, alcohol drinking and esophageal cancer: findings from the JACC Study. *J Epidemiol.* 2005;5:S212–S219. doi:10.2188/jea.15.S212.
31. Wall TL. Genetic association of alcohol and aldehyde dehydrogenase with alcohol dependence and their mechanisms of action. *Ther Drug Monit.* 2005;27:700–703. doi:10.1097/01.ftd.0000179840.78762.33.
32. Carr LG, Foroud T, Stewart T, Castelluccio P, Edenberg HJ, Li TK. Influence of ADH1B polymorphism on alcohol use and its subjective effects in a Jewish population. *Am J Med Genet.* 2002;112:138–143. doi:10.1002/ajmg.10674.
33. Monzoni A, Masutti F, Saccoccio G, Bellentani S, Tiribelli C, Giacca M. Genetic determinants of ethanol-induced liver damage. *Mol Med.* 2001;7:255–262.
34. The sixth report of the Joint National Committee on Prevention. Detection, evaluation, and treatment of high blood pressure. *Arch Intern Med.* 1997;157:2413–2446. doi:10.1001/archinte.157.21.2413.
35. Bellentani S, Saccoccio G, Costa G, et al. Drinking habits as cofactors of risk for alcohol induced liver damage. The Dionysos Study Group. *Gut.* 1997;41:845–850.
36. Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *Br Med J (Clin Res Ed).* 1986;292:13–15.
37. Osawa H, Mori Y. Sonographic diagnosis of fatty liver using a histogram technique that compares liver and renal cortical echo amplitudes. *J Clin Ultrasound.* 1996;24:25–29. doi:10.1002/(SICI)1097-0096(199601)24:1<25::AID-JCU4>3.0.CO;2-N.
38. Agarwal DP. Cardioprotective effects of light-moderate consumption of alcohol: a review of putative mechanisms. *Alcohol Alcohol.* 2002;37:409–415.
39. Becker U, Denis A, Sorensen TI, et al. Prediction of risk of liver disease by alcohol intake, sex, and age: a prospective study. *Hepatology.* 1996;23:1025–1029. doi:10.1002/hep.510230513.
40. Harrison SA, Kadakia S, Lang KA, Schenker S. Non alcoholic steatohepatitis: what we know in the new millennium. *Am J Gastroenterol.* 2002;97:2714–2724.
41. Omagari K, Kadokawa Y, Masuda J, et al. Fatty liver in non alcoholic non overweight Japanese adults. Incidence and clinical characteristics. *J Gastroenterol Hepatol.* 2002;17:1098–1105. doi:10.1046/j.1440-1746.2002.02846.x.

日本人の食事摂取基準(2010年版)の策定の概要

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日本人の食事摂取基準(2010年版)の策定の概要*

keywords：食事摂取基準、総論、活用

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昨年(2009年)、厚生労働省から「日本人の食事摂取基準(2010年版)」が発表された。2010年版は2005年版で示された考え方が踏襲されているが、数値の時代から理論・理屈の時代に、そして、活用は数値をあてはめる時代から考える時代に入ったという印象を強く受ける記述になっている。食事摂取基準の基本的な考え方はほとんどが「総論」で記述されている。「総論」の特徴をあげるとすれば、「活用の基礎理論」が盛り込まれたこと、活用目的が3種類に分けられて記述されたこと、そして、アセスメントの重要性が強調されたことであろう。これで現場が食事摂取基準をじゅうぶんに活用できるかといえば、そこまでは至っていない印象が強いが、それでも、栄養管理業務が医療業務のひとつであり、「科学」であるとすれば、食事摂取基準の理論、特に、総論の内容は栄養管理に携わる者が必ず理解していなければならないことは明らかである。

1. はじめに

昨年(2009年)、厚生労働省から「日本人の食事摂取基準(2010年版)」が発表された。これは厚生労働省のホームページ上に全文が掲載されていて、pdfファイルとしてダウンロードすることができるので、ぜひ、ご覧いただきたい(http://www.mhlw.go.jp/bunya/kenkou/sessyu-kijun.html)。「日本人の食事摂取基準(2010年版)」は全306ページから構成されている。これだけ大量の情報を正確に読み、理解し、活用するのは至難の業だと思われる。そこで、どこがエッセンスであり、どこに力を入れて読めば、正しく理解し、正しく活用できるかについて考えてみることにしたい。

なお、この文章は、「日本人の食事摂取基準(2010年版)」を読まずに済ませたい読者を対象とした、「日本人の食事摂取基準(2010年版)」の紹介文ではないため、あらかじめ注意をされたい。

2. 何よりも「総論」が大切

全体は「総論」と「各論」に分かれている。食事摂取基準の考え方の基本がすべて「総論」で説明されているので、どの栄養素(エネルギーも含む)に興味をもっているか、どの栄養素(エネルギーも含む)についての情報を必要としているかにかかわらず、総論はていねいに読む必要がある。つまり、読解の順序は、

「総論」→「各論の中で必要とする部分」

となるであろう。

「総論」は、「策定の基礎理論」と「活用の基礎理論」のふたつの部分に分かれている。注意すべきことは、両者とも、基礎的な理論が記述されたものであり、事例集でも指示書でもないことである。つまり、ここに書かれている基礎理論を理解し、それにしたがって、目の前の状況をよく観察し、しっかりと自分の頭を使って考えて食事摂取基準を活用することが求められている。この点でも、2010年版は2005年版の考え方を踏襲し、その考え方や活用方法

*The outline of the Dietary Reference Intakes for Japanese (2010)

をさらに前進させたものと理解できる。ここで大切なことは、「策定の基礎理論」が正しく理解されなければ、「活用の基礎理論」は理解できないということである。したがって、食事摂取基準の使い方(活用)に関する情報を得たいと考える場合にも、「策定の基礎理論」の正しい理解が前提となる。

ところで、「日本人の食事摂取基準(2010年版)」の基本中の基本は、やはり、5種類(エネルギーを含めれば6種類)の指標の意味と目的を正しく理解することであろう。2005年版とほとんど変更はないが、栄養素については基本的な概念をまとめた表が添えられており、理解に役立つであろう(表)。ここでも、指標の名称の丸暗記ではなく、それぞれの指標がもつ意味を深く理解することの大切さが強調されている。

つまり、食事摂取基準は数値の時代から、理論・理屈の時代に、そして、活用は、数値をあてはめる時代から考える時代に入ったと言ってよいであろう。

表　栄養素の指標の概念と特徴のまとめ　日本人の食事摂取基準(2010年版)から一部抜粋

目的	摂取不足による健康障害からの回避	摂取過剰による健康障害からの回避	生活習慣病の一次予防
指標	推定平均必要量、推奨量、目安量	耐容上限量	目標量
値の算定根拠となる主な研究方法	実験研究、疫学研究(介入研究を含む)	症例報告	疫学研究(介入研究を含む)
健康障害が生じるまでの典型的な摂取期間	数か月間	数か月間	数年～数十年間
通常の食品を摂取している場合に注目している健康障害が発生する可能性	ある	ほとんどない	ある
サプリメントなど、通常以外の食品を摂取している場合に注目している健康障害が発生する可能性	ある(サプリメントなどには特定の栄養素しか含まれないため)	ある(厳しく注意が必要)	ある(サプリメントなどには特定の栄養素しか含まれないため)
算定された値を守るべき必要性	可能な限り考慮する(回避したい程度によって異なる)	必ず考慮する	関連するさまざまな要因を検討して考慮する
算定された値を守った場合に注目している健康障害が生じる可能性	推奨量付近、目安量付近であれば、可能性は低い	上限量未満であれば、可能性はほとんどないが、完全には否定できない	ある(他の関連要因によっても生じるため)

3.「活用の基礎理論」が示すもの

今回の食事摂取基準で初めて、「活用」を強く意識した記述がなされるようになった。栄養所要量と呼ばれていたころも含めて、食事摂取基準が本来、使うべきガイドラインであることを考えれば当たり前のことである。「活用の基礎理論」で特に強調されていることは次の4点であろう。

①対象者の明確化(疾患を有する者も含む)

狭義には「健康な個人、ならびに、健康な人を中心として構成されている集団」とあるが、「何らかの軽度な疾患(例えば、高血圧、脂質異常症、高血糖)を有していても自由な日常生活を営み、当該疾患に特有の食事指導、食事療法、食事制限が適用されたり、推奨されたりしていない者を含む」とされている。さらに、「特有の食事指導、食事療法、食事制限が適用されたり、推奨されたりする疾患を有する場合、または、ある疾患の予防を目的として特有の食事指導、食事療法、食事制限が適用されたり、推奨されたりする場合には、その疾患に関連する治療ガイドライン等の栄養管理指針を優先して用いるとともに、食事摂取基準を補助的な資料として参照することが勧められる」とある。このことは、疾患を有する者、すなわち、入院中の患者や、外来へ通院している患者に用いるガイドラインのひとつとして食事摂取基準を位置づけており、臨床栄養分野の栄養士、管理栄養士にとっても食事摂取基準が重要なガイドラインのひとつであることを示しているものと考えられる。

②活用目的の明確化

食事摂取基準を活用する主な目的として「食事改善」と「給食管理」の2つをあげ、さらに、前者を「対象者を個人として扱う場合」と「集団として扱う場合」に分けて、それぞれについての理論が説明されている。食事摂取基準を用いる者は、この中のどれを目的として用いるのかを明らかにしたうえで、その理論に基づいて用いることが勧められている。

③アセスメントの重要性

上記のどの目的に用いる場合においても、アセスメントの重要性が強調されている。

アセスメント→プランニング→実行→評価(アセスメント)→・・・

という無限ループで栄養管理などの業務を行っていくことが勧められている。

④食事アセスメント理論の重要性

食事アセスメント理論への正しい理解と、それに基づく

食事アセスメント結果の正しい解釈の重要性が強調されている。特に、食事アセスメントにおける測定誤差の存在とその程度について具体的な記述があり、食事アセスメントにおける測定誤差に関する知識と理解が食事摂取基準の正しい活用に重要な役割を果たすことが強調されている。

しかしながら、他の章に比べると、この章の参考文献はかなり少ない。これは、この章の信頼度が他の章に比べて低いのではないかということを示しており、食事摂取基準を使う側からすれば、不安材料である。そして、同時に、この分野の研究や調査が不足しており、それを推進しなければならないことを示していると理解できるだろう。

4. 演習問題

総論で述べられている「理論・理屈」が、食事摂取基準を正しく使う（活用する）上で大切であることを理解し、自分の食事摂取基準の理解度がどの程度であるかを確認していただくことを目的として、演習問題を作ってみた。自信のある人は、「日本人の食事摂取基準（2010年版）」を読まずに、自信があまりないか、いままでに食事摂取基準についてあまり学んだ経験がない人は「日本人の食事摂取基準（2010年版）」を一通りお読みいただいた後に、解答していただきたい。管理栄養士・栄養士の友人や同僚と意見交換をしたり、先輩や先生の意見を求めたりするのもよいかもしれない。

解答は、（ほぼ正しい）、（ほぼ誤り）のいずれかである。ヒントを参考にしていただくのもよいかもしれない。

Q1 推定エネルギー必要量を習慣的に摂取していればほぼ太りもやせもしないと考えてよい。

（ヒント）食事摂取基準の特徴のひとつである「確率的な考え方」を正しく理解しているかどうかを問う問題である。

Q2 通常の食品だけを用いている場合、たんぱく質の推奨量を超えた献立を作ることは「たんぱく質の食事摂取基準からみて」悪いことではない。

（ヒント）「推奨量」の定義を正しく理解できているかどうか、摂取量と摂取不足確率との関係を表す図を正しく理解できているかどうかを問う問題である。

Q3 55歳女性。骨折予防のためには、カルシウムは余裕をみて650mg/日くらいよりも850mg/日くらい食べるほうがよい。

（ヒント）これも、「推奨量」の定義を正しく理解できているかどうか、摂取量と摂取不足との関係を表す図を正しく理解できているかどうかを問う問題である。

Q4 ある日の給食の献立のビタミンAが耐容上限量を超えていた（注意：耐容上限量は2005年版における上限量と同じ意味である）が、この献立に問題はない。

（ヒント）食事摂取基準の特徴のひとつである「習慣」についての問題である。

Q5 サプリメントを使っていない人でも耐容上限量には気をつけるべきである。

（ヒント）サプリメントと耐容上限量の2つが、「摂取量」を通して正しく理解できているかどうかを問う問題である。

Q6 食事摂取基準は病気をもっている人は対象としていない。

（ヒント）食事摂取基準の対象者に関する基本的な問題である。

Q7 習慣的な摂取量が目安量を下回っていたら、不足していると考えられる。

（ヒント）目安量の定義を正しく理解できているかどうかを問う問題である。

Q8 一般的にいって、成人の推奨量と小児の推奨量はほぼ同じくらいの精度をもっている。

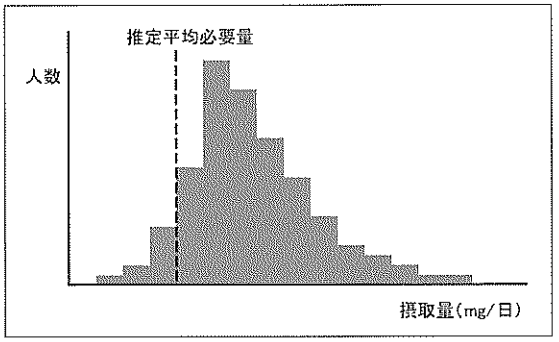
（ヒント）小児の食事摂取基準の数値がどのように算定されているかに関する知識を問う問題である。

Q9 推奨量と目標量はほぼ同じ期間の習慣的な摂取量を考えて算定されている。

（ヒント）「習慣的な摂取量」の「習慣的」が示す意味は指標によって異なることを正しく理解できているかどうかを問う問題である。

Q10 1日間の秤量食事記録法を用いて、ある集団のある栄養素の摂取量を調べた。摂取量の分布が図のようになった。真の不足者数はこの方法で得られる不足者数よりも多い。

（ヒント）食事調査における申告誤差に関する知識を実際に即して理解できているかどうかを問う問題である。



図

Q11 たんぱく質には耐容上限量が設定されていない。このことは、アミノ酸サプリメントの安全性を保証していると考えてよい。

（ヒント）「耐容上限量が設定されていない」ことが示す意味を正しく理解できるかどうかを問う問題。

Q12 ビタミンCの習慣的な摂取量が推定平均必要量付近であると、およそ50%の確率で、ビタミンC欠乏症である壊血病に罹ると考えられる。

（ヒント）どのような状態をもって「不足」とするかは栄養素によって異なる。ビタミンCが「不足」するのはどのような状態のときかについての知識を問う問題。

Q13 職場の給食施設では、食べに来ている人をひとりずつ調査できない場合が多い。このような給食施設では、性・年齢階級、身体活動レベルを考慮した給食献立の作成は無理である。

（ヒント）食事摂取基準では、対象者のアセスメントを行い、その結果に基づいて給食計画を立てることを勧めているが、「アセスメント」とは何かについてじゅうぶんに理解できているかどうかを問う問題。

5. 解答例

解答例を作ってみた。ただし、あくまでも著者の解釈であって、正解とは限らない。「日本人の食事摂取基準(2010年版)」をしっかりとお読みいただき、栄養士・管理栄養士の友人や同僚と意見交換をしたり、先輩や先生の意見を求めたりして、自分なりの解答を作っていただければと思う。

A1 たとえば、同じ性、年齢階級、身体活動レベルの人が100人いた場合、それぞれの人のエネルギー必要量は少しずつ異なる。その平均値がこの値だろうという推定値が推定エネルギー必要量です。それを個人に戻して考えると、その人の必要量を測定できない場合、推定値としてもっとも確からしい値が推定エネルギー必要量といえる。しかし、その人の本当の必要量はこの値とは異なるから、推定必要量を摂取すれば、体重は増えるか、または減るであって、体重が保たれるわけではない。どうなるかは食べてみないとわからない（食べてみればわかる）。

A2 推奨量程度のたんぱく質を摂取していれば、たんぱく質の不足はほぼだれにも起こらないと考えられる。それ以上を摂取しても、同じく、ほぼだれにも不足は起こらないと考えられる。したがって、不足を避けるという観点からは両者にそれほど大きなちがいはない。一方、通常の食品だけからたんぱく質を摂取している限り、過剰摂取による健康障害が起こるほど大量に摂取するとはほとんど考えられない。たんぱく質が多い食事は脂質も多く、また、価格も高くなりやすいといった問題が生じやすいかもしれないが、この問題では、「悪いことではない」と答えるのが正しいであろう。

A3 食事摂取基準では、カルシウムには推定平均必要量と推奨量が示されていて、この対象者における推奨量は650mg/日であり、この摂取量であれば、およそ97.5%の女性で不足していないことが示されている。850mg/日を摂取すれば不足による健康障害のリスクはさらに下がるが、新たにその恩恵を受ける人はわずかに2%程度で、残りの人には新たなメリットはない。これらのことから、「良いことはそれほどない」と考えるのが正しいであろう。

A4 ビタミンAは食品によってその含有量が大きく異なる代表的な栄養素である。献立によってはビタミンAが耐容上限量を上回ってしまうことがあるかもしれない。しかし、食事摂取基準は、習慣的な摂取量についての値であって、1食の中に含まれる栄養素量の過不足を判断するためのものではない。したがって、この献立には問題はないと考えられる。

A5 断言はできないかもしれないが、通常の食品だけを摂取している（サプリメントも強化食品を使っていない）人の場合、すべての栄養素について、習慣的な摂取量が耐容上限量を超えるような食べ方になる可能性は極めて低い。したがって、サプリメントを使っていない人の場合は、事実上、耐容上限量には気をつけなくてもよいと考えられる。

A6 有病者も食事摂取基準を用いる対象者に入る。ただし、その病気のための特別の食事管理を必要とする場合は、その食事管理が食事摂取基準よりも優先される。しかし、病気をもっていても、その病気に特別の食事管理が求められていない栄養素については、食事摂取基準に従うことになり、また、特別の食事管理を必要とはしない病気の場合には、健康な人と同じように食事摂取基準を用いるのが正しいであろう。

A7 目安量は、不足が観察されない集団におけるその栄養素の摂取量の中央値として与えられる。不足している人がいない集団であるから、中央値ではなくて最低値を選んでもよいはずであるが、他の集団の中に、必要量がもっと多い人がいるかもしれない。その人に対しても不足しないであろう数値として中央値が用いられる（中央値がこの目的にもっとも適した指標というわけではないが、他に適切な指標が存在しないという理由によるのであろう）。これは、その栄養素を摂取量が目安量を下回っていても「不足していない」可能性がかなりあることを示している。つまり、目安量よりも摂取量が少なくても「不足している」という判断はできない。逆に、目安量よりも摂取量が多い場合は、「不足している可能性はほとんどない」といえる。

A8 食事摂取基準で参考になる研究のほとんどは成人を対象に行われる。特に、推定平均必要量を決めるための出納実験を小児で行うのは研究倫理上、困難である。そのため、成人で実験を行って値を定め、次に、身体の大きさのちがいや成長による付加的な必要量などを考慮して、小児の数値を推定する。したがって、小児の数値は成人の数値に比べて信頼度は総じて低いと考えるべきであろう。

A9 出納実験を行って必要量を測定した場合、はじめに推定平均必要量を求め、その次に、実験で観察された必要量の個人差（必要量の分布幅）を用いて推奨量を求める。さらに、必要量の個人差の分布幅を正確に測定できた栄養素はそれほど多くなく、多くの栄養素群でひとつの値を暫定的に用いているのが実情である。したがって、推定平均必要量のほうが推奨量よりも信頼度の高い数値であろうと考えられる。

A10 食事記録法をはじめ、ほとんどの食事調査法で過小申告が認められる。次に、1日間の摂取量の分布は習慣的な摂取量の分布よりも広くなる。したがって、この2つの問題を考慮すると、真の習慣的な摂取量の分布は、この図よりも全体として右にずれ、かつ、幅が狭いものと推定される。このことから、真の不足者数は、この図から推定される不足者数よりも少ないものと予想される。

A11 「耐容上限量が設定されていない」のは、過剰摂取による明確な健康障害の報告が文献上、見いだされなかったことを示すだけであり、無限に摂取しても安全である（健康障害は生じない）ことを示すものではない。したがって、アミノ酸サプリメントの安全性を保証しているわけではない。

A12 ビタミンCの推定平均必要量は、その血漿濃度で決められている。しかし、壊血病ではなく、心臓血管系の疾病予防効果ならびに有効な抗酸化作用が期待できる濃度が用いられている。この濃度は壊血病を予防する濃度よりも高いから、推定平均必要量付近を摂取していても壊血病が50%の確率で発症するわけではない。

A13 職場の給食施設利用者の性・年齢の分布や、利用者がどの食事を選択し、摂取しているかを知るのは困難な場合が多い。しかし、その職場の職員構成(性・年齢の分布)に関する情報はほとんどの職場で存在するであろう。また、職員の職務内容から身体活動レベルの分布を推定することも、限界はあるが、不可能ではない。したがって、あくまでも限定付きではあるが、これらの情報(これもアセスメントのひとつである)を給食献立の作成に活用することが考えられる。

6. おわりに

「日本人の食事摂取基準(2010年版)」は2005年版と比べて、それほど大きく変わってはいない。むしろ、2005年版で示された考え方を踏襲し、さらに、それを推し進めたものと理解できる。そして、2005年版では、じゅうぶんに踏み込めていなかった点や、あいまいであった記述に対して、少しではあるにせよ、ていねいかつ明確な説明が試みられている。この点に注意して、読んでいただければ、2010年版の真価を理解していただけることと思う。

繰り返しになるが、食事摂取基準の考え方と、活用時に注意すべき事柄の多くは、「総論」で説明されている。総論をていねいに読み、そこに書かれていることを完全に理解すること、それが食事摂取基準を正しく理解し、正しく活用するための唯一、最良、最短の方法であることをご理解いただきたい。

推薦図書

食事摂取基準の基本的な考え方や活用について理解するためには、『佐々木敏. 食事摂取基準入門--そのこころを読む--. 同文書院, 2010』を、食事摂取基準の理論的基礎概念であるEBNや確率論など、疫学に関連する部分の知識を得るためには、『佐々木敏. わかりやすいEBNと栄養疫学. 同文書院, 2005』を読まれることをお勧めする。日本人の食事摂取基準(2010年版)はこれらに書かれている基本事項を理解しているものとして書かれている点に注意が必要であろう。

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Applied nutritional investigation

Neighborhood socioeconomic status in relation to dietary intake and insulin resistance syndrome in female Japanese dietetic students

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Abstract

Objective: An increasing number of studies in Western countries have shown that living in a socioeconomically disadvantaged neighborhood is associated with unfavorable dietary intake patterns and health status. However, information on such neighborhood socioeconomic differences in diet and health among different cultural settings, including Japan, is limited. This cross-sectional study examined the association of neighborhood socioeconomic status (SES) with dietary intake and a summary score of the insulin resistance syndrome (IRS) in a group of young Japanese women.

Methods: Subjects were 1081 female Japanese dietetic students aged 18 to 22 y residing in 295 municipalities in Japan. Neighborhood SES index was defined by seven municipal-level variables, namely unemployment, household overcrowding, poverty, education, income, home ownership, and vulnerable group, with an increasing index signifying increasing neighborhood socioeconomic disadvantage. Dietary intake was estimated using a validated, comprehensive self-administered diet-history questionnaire. Measurements of body mass index, systolic blood pressure, fasting high-density lipoprotein cholesterol, triacylglycerol, glucose, and insulin were combined into an IRS score, with an increasing score signifying increasing levels of components of the IRS.

Results: Neighborhood SES index was not associated with most of the dietary variables, body mass index, high-density lipoprotein cholesterol, triacylglycerol, or glucose. However, neighborhood SES index was significantly positively associated with systolic blood pressure, insulin, and IRS score, after adjustment for potential confounding or mediating factors, including household SES, dietary, and lifestyle factors.

Conclusion: Neighborhood socioeconomic disadvantage was associated with unfavorable profiles of the IRS score, but not dietary intake, in a group of young Japanese women.

Keywords: Neighborhood socioeconomic status; Diet; Insulin resistance syndrome; Young women; Japan; Epidemiology

Introduction

Because living conditions are shaped by characteristics of the residential environment, neighborhood characteristics may have some impact on lifestyle factors and, hence, on health status, beyond any effect of the characteristics of the individual. In fact, an increasing number of studies conducted in Western countries have shown that living in a socioeconomically disadvantaged neighborhood is associated with unfavorable dietary intake patterns [1–4] and an unfavorable profile of the insulin resistance syndrome (IRS) [5–11].

However, information on the relation of neighborhood socioeconomic status (SES) with diet and health in other countries is sparse, including Japan. Given the unclear or even inverse association between individual SES and health outcomes observed in the Japanese [12–14] vis-à-vis the

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consistent positive associations of individual and neighborhood SES with diet and health in Western populations [1–11,15–17], the association of neighborhood SES with dietary intake and health outcomes may differ between Western countries and Japan. In a study of young Japanese women, for example, neighborhood SES was not materially associated with dietary intake, but increasing neighborhood socioeconomic disadvantage was associated with increasing body mass index (BMI) [18].

In the present study, we examined the association of a neighborhood SES index, recently formulated for Japanese conditions [19], with selected dietary variables, as assessed using a previously validated, comprehensive self-administered diet-history questionnaire (DHQ) [20–23], and a summary score of the IRS, developed by Diez Roux et al. [5], in a group of young Japanese women. We hypothesized that neighborhood socioeconomic disadvantage is associated with unfavorable profiles of the IRS score, but not dietary intake.

Materials and methods

Study sample

This observational cross-sectional study was conducted from February to March 2006 and from January to March 2007 in female Japanese dietetic students from 15 institutions in Japan ($n = 1176$). A detailed description of the study design and survey procedure has been published elsewhere [24,25]. Written informed consent was obtained from each participant and from a parent for participants younger than 20 y. The study protocol was approved by the ethics committee of the National Institute of Health and Nutrition, Japan.

In Japan, the 2372 municipalities consisted of 164 wards, 736 cities, 1178 towns, and 294 villages (as of October 1, 2005) [26]. We used municipalities as proxies for neighborhoods [18,27], which is in accordance with previous Western studies where some administrative divisions are used as proxies for neighborhoods [1–11]. Study participants were linked to their municipalities using their home addresses.

For analysis, we selected women aged 18 to 22 y ($n = 1154$). We then excluded women with previously diagnosed diabetes, hypertension, or cardiovascular disease ($n = 1$) and those with missing information on the variables used ($n = 72$). The final analysis sample consisted of 1081 women who resided in 295 municipalities in Japan.

Neighborhood SES index

We constructed a neighborhood SES index at the municipality level [18,27] using seven variables determined by a factor analysis [19]. These variables were unemployment (percentage of unemployed persons ≥15 y old), household overcrowding (average floor space per residential dwelling), poverty (number of households receiving public assistance per 1000 households), education (percentage of persons 20–64 y old who had completed college or university), income (total taxable income divided by total population), home ownership (percentage of owned houses to total residential households), and vulnerable groups (percentage of households of single persons ≥65 y old to total households) [19]. Data were derived from the 2005 census [26] and other governmental surveys [26,28,29]. These seven variables were combined into a neighborhood summary score (i.e., neighborhood SES index) constructed by summing *Z*-scores for each of the seven variables (for unemployment, poverty, income, and vulnerable groups, data were log-transformed before calculating *Z*-scores; *Z*-scores for household overcrowding, education, income, and home ownership were multiplied by −1 before summing), with a higher neighborhood SES index signifying increasing neighborhood socioeconomic disadvantage [19]. A detailed description of the neighborhood SES index has been published elsewhere [18].

Dietary intake

Dietary habits during the preceding month were assessed using a comprehensive self-administered DHQ. Details of the DHQ's structure, calculation of dietary intake, and validity for commonly studied nutritional factors have been published elsewhere [20–25]. Briefly, the DHQ is a structured 16-page questionnaire that asks about the consumption frequency and portion size of selected foods commonly consumed in Japan and the general dietary behavior and usual cooking methods [20]. Estimates of daily intake for foods (150 items in total), energy, and selected nutrients were calculated using an ad hoc computer algorithm for the DHQ [20,23] based on the *Standard Tables of Food Composition in Japan* [30]. Dietary variables examined in this study were dietary energy density [24] and potential renal acid load [25], variables significantly associated with several metabolic risk factors in the present population, and their main determinants (energy-providing nutrients, dietary fiber, minerals, and selected food groups). To minimize the influence of dietary misreporting, an ongoing controversy in studies that collect dietary information using self-report instruments [31], dietary variables were energy adjusted using the density method (except for energy density).

IRS score

A summary score of IRS developed by Diez Roux et al. [5] was calculated based on measurements of BMI, systolic blood pressure, fasting high-density lipoprotein cholesterol, triacylglycerol, glucose, and insulin. Detailed descriptions of the measurement of each component have been published elsewhere [24,25]. These six variables were combined into a summary IRS score constructed by summing Z-scores for each of the six variables (for triacylglycerol and insulin, data were log-transformed before calculating *Z*-scores; Z-scores for high-density lipoprotein cholesterol were multiplied by −1 before summing), with an increasing score signifying increasing levels of components of the IRS. A detailed description of the IRS score has been provided by Diez Roux et al. [5].

Other variables

Based on the reported home address, each participant was grouped into one of six regions (Hokkaido and Tohoku; Kanto; Hokuriku and Tokai; Kinki; Chugoku and Shikoku; or Kyushu) and into one of three municipality levels (ward; city; or town and village). The participant was also grouped into one of four institution types (4-y private, 2-y private, 4-y public, or 2-y public) based on the institution she attended and into one of three living statuses (living with family, living alone, or living with others), as self-reported in a lifestyle questionnaire.

Table 1
Dietary, metabolic, and lifestyle characteristics of 1081 Japanese women aged 18 to 22 y*

Variable	
Food intake (g/1000 kcal)	
Cereals and potatoes	241.3 (237.9–244.7)
Confectioneries and sugars	40.2 (39.1–41.3)
Fats and oils	11.8 (11.4–12.1)
Fruits and vegetables	179.5 (173.9–185.1)
Fish, meat, and eggs	81.7 (80.1–83.3)
Dairy products	91.0 (86.7–95.3)
Nutrient intake	
Protein (percent energy)	13.5 (13.4–13.6)
Fat (percent energy)	29.2 (28.9–29.5)
Carbohydrate (percent energy)	55.7 (55.4–56.1)
Dietary fiber (g/1000 kcal)	6.9 (6.7–7.0)
Phosphorus (mg/1000 kcal)	517 (511–522)
Potassium (mg/1000 kcal)	1111 (1096–1127)
Calcium (mg/1000 kcal)	283 (277–289)
Magnesium (mg/1000 kcal)	121 (119–123)
Dietary energy density (kcal/g)	1.41 (1.40–1.43)
Potential renal acid load (mEq/1000 kcal; measurement of diet-induced acid–base load)	5.90 (5.66–6.16)
Body mass index (kg/m^2)	21.4 (21.2–21.5)
Systolic blood pressure (mmHg)	106.3 (105.6–106.9)
High-density lipoprotein cholesterol (mg/dL)	70.8 (70.0–71.5)
Triacylglycerol (mg/dL)†	56.2 (54.8–57.5)
Glucose (mg/dL)	84.0 (83.7–84.4)
Insulin (ng/mL)†	7.4 (7.2–7.7)
Current smokers	2.3
Current alcohol drinkers	41.9
Physical activity (total metabolic equivalent-hours/d)	33.9 (33.8–34.0)

* Values are means (95% confidence intervals) or percentages of participants.
† Calculated using back-transformation of natural-log transformed values.

Current smoking (yes or no) and current alcohol drinking (yes or no) were self-reported in the lifestyle questionnaire and DHQ, respectively. Physical activity was computed as the average metabolic equivalent-hours score per day on the basis of the frequency and duration of five activities (sleeping, high- and moderate-intensity activities, walking, and sedentary activities) over the preceding month, as reported in the lifestyle questionnaire [24].

Statistical analysis

All statistical analyses were performed using SAS 9.1 (2003, SAS Institute Inc, Cary, NC, USA). Using the PROC GLM procedure, linear regression models were constructed to examine the association of neighborhood SES index with dietary variables and the summary IRS score and its components. For analyses, participants were categorized into quartiles according to neighborhood SES index. Multivariate-adjusted mean values (with 95% confidence intervals) of dietary variables and the summary IRS score and its components were calculated by quartile of neighborhood SES index. Potential confounding or mediating factors included in the multivariate models were survey year [24,25,27], household SES variables, i.e., institution type [18,27,32] and living status [18,27,33], and non-dietary lifestyle factors, i.e., current smoking status, current alcohol drinking status, and physical activity [18]. In the analysis of the summary IRS score and its components, dietary factors, i.e., dietary energy density [24] and potential renal acid load [25], were also included. In addition, geographical variables, i.e., region [18,27] and municipality level [18,27], were included, considering regional or urban–rural differences in neighborhood SES in Japan [19,34,35], although this may be an over-adjustment. We tested for linear trends with increasing levels of neighborhood SES index by assigning each participant the median value for the category and modeling this value as a continuous variable. All reported P values are two-tailed, and $P < 0.05$ was considered statistically significant. Because the great majority of municipalities had only a few study participants (median 1, interquartile range 1–3), no special methods were needed to account for within-neighborhood correlations in outcomes [4,5,18,27].

Results

Dietary, metabolic, and lifestyle characteristics are listed in Table 1. Mean dietary intakes of macronutrients were 13.5% of energy for protein, 29.2% of energy for fat, and 55.7% of energy for carbohydrate. Mean BMI was 21.4 kg/m^2. Geographic, household SES, and lifestyle characteristics according to quartile of neighborhood SES index are listed in Table 2. Neighborhood SES index was associated with region, municipality level, and institution type. The higher quartiles of neighborhood SES index (increasing neighborhood socioeconomic disadvantage) included more participants living in Hokkaido and Tohoku, Chugoku and Shikoku, and Kyushu and fewer participants living in Kanto, Hokuriku and Tokai, and Kinki; more participants living in wards and fewer living in towns and villages; and more participants attending 4-y private, 2-y private, and 4-y public institutions and fewer attending 2-y public institutions.

Dietary characteristics according to quartile of neighborhood SES index are presented in Table 3. Neighborhood SES index was not associated with any dietary variables examined after adjustment for possible confounding or mediating factors including household SES and non-dietary lifestyle variables, with the exception of a positive association with cereals and potatoes and a negative association with dairy products, phosphorus, potassium, calcium, and magnesium. Further adjustment for geographic variables did not change the results materially, with the exception of the loss of the above-mentioned associations (data not shown).

Components of IRS and summary IRS score according to quartile of neighborhood SES index are presented in Table 4. Neighborhood SES index was not associated with BMI, high-density lipoprotein cholesterol, triacylglycerol, or glucose. Conversely, higher neighborhood SES index (increasing neighborhood socioeconomic disadvantage) was significantly associated with higher systolic blood pressure, insulin, and IRS score, after adjustment for potential confounding or mediating factors, including household SES, non-dietary lifestyle, and dietary factors. However, these associations disappeared after further adjustment for geographic variables (data not shown).

Table 2
Geographic, household socioeconomic status, and lifestyle characteristics of 1081 Japanese women aged 18 to 22 y according to quartile category of neighborhood socioeconomic status index*

Variable	Quartile 1 ($n = 263$)	Quartile 2 ($n = 277$)	Quartile 3 ($n = 262$)	Quartile 4 ($n = 279$)	$P^{\dagger}$
Neighborhood socioeconomic status index (median)	−3.19	−0.94	0.22	4.07	—
Survey year					0.61
2006	41.4	36.8	47.7	40.1	
2007	58.6	63.2	52.3	59.9	
Region					<0.0001
Hokkaido and Tohoku	0	0.7	0.4	9.0	
Kanto	71.9	66.8	58.8	23.7	
Hokuriku and Tokai	8.8	22.7	19.5	2.5	
Kinki	16.0	7.2	1.5	15.8	
Chugoku and Shikoku	0.4	0.7	0	13.3	
Kyushu	3.0	1.8	19.9	35.8	
Municipality level					<0.0001
Ward	8.8	9.8	7.3	36.9	
City	78.3	89.2	86.6	59.9	
Town and village	12.9	1.1	6.1	3.2	
Institution type					<0.0001
4-y private	70.0	59.9	75.6	73.5	
2-y private	2.7	1.1	16.0	2.5	
4-y public	17.9	7.6	6.9	21.5	
2-y public	9.5	31.4	1.5	2.5	
Living status					0.06
Living with family	67.7	65.3	35.9	68.5	
Living alone	29.3	29.6	60.7	26.5	
Living with others	3.0	5.1	3.4	5.0	
Current smokers	2.3	1.4	3.1	2.5	0.57
Current alcohol drinkers	37.6	46.9	33.2	49.1	0.12
Physical activity (total metabolic equivalent-hours/d)	33.7 (33.4–34.1)	34.0 (33.7–34.3)	33.7 (33.4–34.1)	34.1 (33.7–34.6)	0.21

* Values are percentages of participants or means (95% confidence intervals) unless otherwise indicated.
† For categorical variables, a Mantel-Haenszel chi-square test was used; for continuous variables, a linear trend test was used, with the median value in each quintile category as a continuous variable in linear regression.

Discussion

As hypothesized, we found in this cross-sectional study of a group of young Japanese women that neighborhood SES was not materially associated with dietary intake, but increasing neighborhood socioeconomic disadvantage was associated with unfavorable profiles of the IRS score. To our knowledge, this is the first study to investigate the association of neighborhood SES with dietary intake and the IRS score in a Japanese population.

Inconsistent with several Western studies [1–4], but consistent with a previous Japanese study [18], we saw no material association between neighborhood SES and dietary variables. The reason for the present finding is unknown. Considering that Japan has long been shown to have lower inequality in individual SES than other developed countries [36], inequalities in neighborhood SES in Japan may be too low to have a measurable influence on dietary habits. Alternatively, the homogenous characteristics of participants in terms of individual SES (i.e., Japanese female dietetic students aged 18–22 y) may have hampered the identification of any meaningful association between neighborhood SES and dietary intake.

Consistent with previous Western studies [5–11], however, we did identify an association between neighborhood socioeconomic disadvantage and an adverse IRS score profile. There are several proximate mechanisms through which neighborhood characteristics could be hypothesized to influence the development of components of IRS [5]. Neighborhood SES may be related to components of IRS by an influence on behaviors linked to diet and physical activity, both of which may be related to insulin resistance [5,10,37]. This is unlikely in the present study, however, because the association between neighborhood SES and the IRS score remained after adjustment for physical activity and dietary factors.

Neighborhood SES may also be related to components of IRS through chronic stress, on the basis that, although sources of chronic stress (such as noise, violence, poverty, vigilance, threat, and alarm) are likely to vary across neighborhoods, chronic stress may be related to the development of components of IRS through endocrine pathways involving the hypothalamo–pituitary–adrenal axis or activation of the sympathetic nervous system [5,9,10,38,39]. Our results are consistent with this environmental stress theory for the role of the social environment in components of IRS, although

Table 3
Dietary characteristics of 1081 Japanese women aged 18 to 22 y according to quartile category of neighborhood socioeconomic status index*

Variable	Quartile 1 (n = 263)	Quartile 2 (n = 277)	Quartile 3 (n = 262)	Quartile 4 (n = 279)	$P^{\dagger}$
Food intake (g/1000 kcal)					
Cereals and potatoes	236.9 (230.1–243.8)	236.5 (229.5–243.5)	244.7 (237.2–252.2)	247.0 (240.2–253.8)	0.02
Confectioneries and sugars	40.6 (38.4–42.7)	41.0 (38.8–43.3)	39.9 (37.5–42.3)	39.3 (37.2–41.5)	0.33
Fats and oils	11.8 (11.1–12.4)	11.9 (11.3–12.6)	11.3 (10.6–12.0)	12.0 (11.3–12.6)	0.69
Fruits and vegetables	180.5 (169.2–191.7)	190.0 (178.4–201.6)	172.2 (159.8–184.5)	175.1 (164–186.3)	0.31
Fish, meat, and eggs	83.2 (80.1–86.3)	82.7 (79.5–85.9)	76.2 (72.8–79.6)	84.3 (81.3–87.4)	0.63
Dairy products	94.3 (85.6–103.0)	91.6 (82.6–100.5)	102.4 (92.8–111.9)	76.5 (67.9–85.1)	0.003
Nutrient intake					
Protein (percent energy)	13.6 (13.4–13.8)	13.6 (13.4–13.8)	13.4 (13.1–13.6)	13.4 (13.2–13.6)	0.15
Fat (percent energy)	29.4 (28.8–30.0)	29.5 (28.8–30.1)	28.7 (28.0–29.4)	29.1 (28.5–29.7)	0.34
Carbohydrate (percent energy)	55.5 (54.8–56.2)	55.4 (54.6–56.1)	56.3 (55.5–57.1)	55.8 (55.0–56.5)	0.55
Dietary fiber (g/1000 kcal)	6.9 (6.6–7.1)	7.0 (6.8–7.3)	6.9 (6.6–7.2)	6.6 (6.4–6.9)	0.09
Phosphorus (mg/1000 kcal)	525 (514–536)	522 (511–534)	521 (508–533)	499 (488–510)	0.0005
Potassium (mg/1000 kcal)	1121 (1089–1152)	1131 (1099–1163)	1116 (1082–1150)	1079 (1048–1110)	0.03
Calcium (mg/1000 kcal)	290 (279–302)	287 (275–299)	296 (283–309)	261 (249–272)	0.0002
Magnesium (mg/1000 kcal)	122 (119–126)	122 (118–125)	123 (120–127)	117 (114–120)	0.02
Dietary energy density (kcal/g)	1.41 (1.38–1.44)	1.40 (1.37–1.42)	1.42 (1.39–1.45)	1.42 (1.40–1.45)	0.34
Potential renal acid load (mEq/1000 kcal; measurement of diet-induced acid–base load)	6.06 (5.55–6.57)	5.76 (5.24–6.29)	5.59 (5.03–6.15)	6.21 (5.71–6.72)	0.55

* Values are means (95% confidence intervals). Adjusted for survey year (2006 and 2007), institution type (4-y private, 2-y private, 4-y public, and 2-y public), living status (living with family, living alone, and living with others), current smoking (yes or no), current alcohol drinking (yes or no), and physical activity (total metabolic equivalents-hours/day, continuous). No significant association was observed after further adjustment for region (Hokkaido and Tohoku; Kanto; Hokuriku and Tokai; Kinki; Chugoku and Shikoku; and Kyushu) and municipality level (ward; city; and town and village).

† A linear trend test was used with the median value in each quintile category as a continuous variable in linear regression.

the association remains speculative because no variables associated with chronic stress were included in the present study.

In addition, the association between neighborhood SES and the IRS score may merely reflect geographic differences in the IRS score, because, although neighborhood SES was associated with region and municipality level, the association between neighborhood SES and IRS score disappeared after adjustment for these geographic variables. Alternatively, it is also possible that neighborhood SES contributes to regional and urban–rural differences in IRS score, and that geographic variables are a proxy for unmeasured neighborhood-level factors that covary with those we are investigating.

In this study, neighborhood SES index was not associated with BMI. However, we found in a recent report [18] studying a similar population that increasing neighborhood socioeconomic disadvantage was independently associated with increasing BMI. The reason for these apparently divergent results is unknown. Although the present study was conducted in students studying in a dietetic course for 1 to 4 y (and thus with more nutritional knowledge), the previous study [18] was conducted in freshmen students just entering a dietetic course (and thus with less nutritional knowledge), although, at least for intake of main food groups and macronutrients and BMI, mean values were similar between subjects in the present and previous [18] studies. Freshmen

Table 4
Components of insulin resistance syndrome and insulin resistance syndrome score of 1081 Japanese women aged 18 to 22 y according to quartile category of neighborhood socioeconomic status index*

Variable	Quartile 1 (n = 263)	Quartile 2 (n = 277)	Quartile 3 (n = 262)	Quartile 4 (n = 279)	$P^{\dagger}$
Body mass index (kg/m^2)	21.0 (20.7–21.4)	21.3 (20.9–21.6)	21.7 (21.4–22.1)	21.4 (21.1–21.7)	0.11
Systolic blood pressure (mmHg)	105.2 (104.0–106.5)	105.2 (103.9–106.5)	106.0 (104.6–107.3)	108.5 (107.3–109.7)	<0.0001
High-density lipoprotein cholesterol (mg/dL)	71.0 (69.5–72.6)	71.6 (70.0–73.2)	70.3 (68.6–72.0)	70.1 (68.5–71.6)	0.28
Triacylglycerol (mg/dL)‡	54.4 (52.4–56.4)	54.9 (52.9–56.9)	59.6 (57.6–61.7)	56.0 (54.0–58.0)	0.36
Glucose (mg/dL)	84.2 (83.4–84.9)	84.0 (83.2–84.8)	83.9 (83.1–84.8)	84.0 (83.2–84.8)	0.80
Insulin (ng/mL)‡	7.1 (7.1–7.2)	7.4 (7.3–7.4)	7.3 (7.3–7.4)	7.9 (7.8–7.9)	0.03
Insulin resistance syndrome score	−0.11 (−0.23 to 0.01)	−0.08 (−0.20 to 0.04)	0.08 (−0.05 to 0.21)	0.11 (0.00 to 0.23)	0.004

* Values are means (95% confidence intervals). Adjusted for survey year (2006 and 2007), institution type (4-y private, 2-y private, 4-y public, and 2-y public), living status (living with family, living alone, and living with others), current smoking (yes or no), current alcohol drinking (yes or no), physical activity (total metabolic equivalents-hours/day, continuous), dietary energy density (kcal/g, continuous), and potential renal acid load (mEq/1000 kcal, continuous). No significant association was observed after further adjustment for region (Hokkaido and Tohoku; Kanto; Hokuriku and Tokai; Kinki; Chugoku and Shikoku; and Kyushu) and municipality level (ward; city; and town and village).

† A linear trend test was used with the median value in each quintile category as a continuous variable in linear regression.

‡ Calculated using back-transformation of natural-log transformed values.

students with less nutritional knowledge may have different results regarding the association of neighborhood SES with dietary intakes and health when compared with those observed in dietetic students with more nutritional knowledge.

Several limitations of the present study deserve mention. First, the participants were selected female dietetic students and may have healthier dietary habits and IRS profiles than the general population, although with regard to the intake of fat and carbohydrate, BMI, and systolic blood pressure at least, mean values in the present study were reasonably comparable to those of a representative sample of Japanese women aged 20 to 29 y (28.6% of energy, 56.2% of energy, 20.5 kg/m^2, and 106.9 mmHg, respectively; data not available for other variables) [40]. Thus, our results might not be extrapolated to the general Japanese population. Second, we relied on census-based measurements at the municipality level as proxies for neighborhoods, but these might not correspond to socially defined neighborhoods. In addition, municipality in Japan may be a somewhat large unit of neighborhoods, given that the median population of 295 municipalities was 121 779 (interquartile range 91 437–274 481). Our study is also limited by the use of the neighborhood SES score as an indirect proxy for the specific features of neighborhoods that may be more relevant [5]. Third, we used a self-administered semiquantitative dietary assessment questionnaire for dietary data collection. Although this questionnaire has been well validated [20–25], actual dietary habits were not observed, so the results should be interpreted cautiously. Fourth, we cannot rule out residual confounding. In particular, parents' SES variables were not available, although these may be at least partly reflected by household SES variables. Fifth, the cross-sectional nature of the study hampers the drawing of any conclusions on causal inferences between neighborhood SES and diet and the IRS.

Conclusion

Although no material association was seen between neighborhood SES and dietary intake, increasing neighborhood socioeconomic disadvantage was associated with unfavorable IRS score profiles in a group of young Japanese women. Efforts to reduce inequalities in neighborhood SES may represent an important strategy in improving the health status of individuals.

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References

[1] Diez-Roux AV, Nieto FJ, Caulfield L, Tyroler HA, Watson RL, Szkio M. Neighbourhood differences in diet: the Atherosclerosis Risk in Communities (ARIC) Study. J Epidemiol Community Health 1999;53:55–63.

[2] Dubowitz T, Heron M, Bird CE, Lurie N, Finch BK, Basurto-Davila R, et al. Neighborhood socioeconomic status and fruit and vegetable intake among whites, blacks, and Mexican Americans in the United States. Am J Clin Nutr 2008;87:1883–91.

[3] Shohaimi S, Welch A, Bingham S, Luben R, Day N, Wareham N, et al. Residential area deprivation predicts fruit and vegetable consumption independently of individual educational level and occupational social class: a cross sectional population study in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk). J Epidemiol Community Health 2004;58:686–91.

[4] Stimpson JP, Nash AC, Ju H, Eschbach K. Neighborhood deprivation is associated with lower levels of serum carotenoids among adults participating in the Third National Health and Nutrition Examination Survey. J Am Diet Assoc 2007;107:1895–902.

[5] Diez Roux AV, Jacobs DR, Kiefe CI. Neighborhood characteristics and components of the insulin resistance syndrome in young adults: the Coronary Artery Risk Development In Young Adults (CARDIA) study. Diabetes Care 2002;25:1976–82.

[6] Sundquist J, Malmstrom M, Johansson SE. Cardiovascular risk factors and the neighbourhood environment: a multilevel analysis. Int J Epidemiol 1999;28:841–5.

[7] Dragano N, Bobak M, Wege N, Peasey A, Verde PE, Kubinova R, et al. Neighbourhood socioeconomic status and cardiovascular risk factors: a multilevel analysis of nine cities in the Czech Republic and Germany. BMC Public Health 2007;7:255.

[8] Black JL, Macinko J. Neighborhoods and obesity. Nutr Rev 2008; 66:2–20.

[9] Cozier YC, Palmer JR, Horton NJ, Fredman L, Wise LA, Rosenberg L. Relation between neighborhood median housing value and hypertension risk among black women in the United States. Am J Public Health 2007;97:718–24.

[10] Glass TA, Rasmussen MD, Schwartz BS. Neighborhoods and obesity in older adults: the Baltimore Memory Study. Am J Prev Med 2006; 31:455–63.

[11] Chichlowska KL, Rose KM, Diez-Roux AV, Golden SH, McNeill AM, Heiss G. Individual and neighborhood socioeconomic status characteristics and prevalence of metabolic syndrome: the Atherosclerosis Risk in Communities (ARIC) study. Psychosom Med 2008;70:986–92.

[12] Martikainen P, Ishizaki M, Marmot MG, Nakagawa H, Kagamimori S. Socioeconomic differences in behavioural and biological risk factors: a comparison of a Japanese and an English cohort of employed men. Int J Epidemiol 2001;30:833–8.

[13] Nishi N, Makino K, Fukuda H, Tatara K. Effects of socioeconomic indicators on coronary risk factors, self-rated health and psychological well-being among urban Japanese civil servants. Soc Sci Med 2004; 58:1159–70.

[14] Hirokawa K, Tsutusmi A, Kayaba K. Impacts of educational level and employment status on mortality for Japanese women and men: the Jichi Medical School cohort study. Eur J Epidemiol 2006;21:641–51.

[15] Winkleby MA, Jatulis DE, Frank E, Fortmann SP. Socioeconomic status and health: how education, income, and occupation contribute to risk factors for cardiovascular disease. Am J Public Health 1992; 82:816–20.

[16] Lantz PM, House JS, Lepkowski JM, Williams DR, Mero RP, Chen J. Socioeconomic factors, health behaviors and mortality: results from a nationally representative prospective study of US adults. JAMA 1998;279:1703–8.

[17] Lahelma E, Martikainen P, Laaksonen M, Aittomaaki A. Pathways between socioeconomic determinants of health. J Epidemiol Community Health 2004;58:327–32.

[18] Murakami K, Sasaki S, Okubo H, Takahashi Y, for the Freshmen in Dietetic Courses Study II Group. Neighborhood socioeconomic status in relation to dietary intake and body mass index in female Japanese dietetic students. Nutrition 2009;25:745–52.

[19] Fukuda Y, Nakamura K, Takano T. Higher mortality in areas of lower socioeconomic position measured by a single index of deprivation in Japan. Public Health 2007;121:163–73.

[20] Sasaki S, Yanagibori R, Amano K. Self-administered diet history questionnaire developed for health education: a relative validation of the test-version by comparison with 3-day diet record in women. J Epidemiol 1998;8:203–15.
[21] Sasaki S, Yanagibori R, Amano K. Validity of a self-administered diet history questionnaire for assessment of sodium and potassium: comparison with single 24-hour urinary excretion. Jpn Circ J 1998; 62:431–5.
[22] Sasaki S, Ushio F, Amano K, Morihara M, Todoriki T, Uehara Y, et al. Serum biomarker-based validation of a self-administered diet history questionnaire for Japanese subjects. J Nutr Sci Vitaminol 2000; 46:285–96.
[23] Murakami K, Sasaki S, Takahashi Y, Okubo H, Hirota N, Notsu A, et al. Reproducibility and relative validity of dietary glycaemic index and load assessed with a self-administered diet-history questionnaire in Japanese adults. Br J Nutr 2008;99:639–48.
[24] Murakami K, Sasaki S, Takahashi Y, Uenishi K, for the Japan Dietetic Students' Study for Nutrition and Biomarkers Group. Dietary energy density is associated with body mass index and waist circumference, but not with other metabolic risk factors, in free-living young Japanese women. Nutrition 2007;23:798–806.
[25] Murakami K, Sasaki S, Takahashi Y, Uenishi K, for the Japan Dietetic Students' Study for Nutrition and Biomarkers Group. Association between dietary acid–base load and cardiometabolic risk factors in young Japanese women. Br J Nutr 2008;100:642–51.
[26] Statistics Bureau, Ministry of Internal Affairs and Communications, Japan. E-Stat (in Japanese). Available at: http://www.e-stat.go.jp/SG1/estat/eStatTopPortal.do. Accessed August 6, 2008.
[27] Murakami K, Sasaki S, Takahashi Y. Uenishi K, for the Japan Dietetic Students' Study for Nutrition and Biomarkers Group. Neighborhood socioeconomic disadvantage is associated with higher ratio of 24-hour urinary sodium to potassium in young Japanese women. J Am Diet Assoc 2009;109:1606–11.
[28] Statistics Bureau, Ministry of Internal Affairs and Communications, Japan. System of social and demographic statistics of Japan. Basic data by municipality (1980–2005) (in Japanese). Tokyo: Statistics Bureau, Ministry of Internal Affairs and Communications, Japan; 2007.
[29] Society for the Study of Municipal Taxation. Indicators of citizen's income 2005 (in Japanese). Tokyo: JPS Co Ltd; 2006.
[30] Science and Technology Agency. Standard tables of food composition in Japan, fifth revised and enlarged edition (in Japanese). Tokyo: Printing Bureau of the Ministry of Finance; 2005.
[31] Murakami K, Sasaki S, Takahashi Y, Uenishi K, Yamasaki M, Hayabuchi H, et al. Misreporting of dietary energy, protein, potassium and sodium in relation to body mass index in young Japanese women. Eur J Clin Nutr 2008;62:111–8.
[32] Karvonen S, Rimpela A. Socio-regional context as a determinant of adolescents' health behaviour in Finland. Soc Sci Med 1996;43:1467–74.
[33] Murakami K, Sasaki S, Okubo H, Takahashi Y, Hosoi Y, Itabashi M. Monetary costs of dietary energy reported by young Japanese women: association with food and nutrient intake and body mass index. Public Health Nutr 2007;10:1430–9.
[34] Fukuda Y, Nakamura K, Takano T. Municipal socioeconomic status and mortality in Japan: sex and age differences, and trends in 1973–1998. Soc Sci Med 2004;59:2435–45.
[35] Fukuda Y, Nakamura K, Takano T. Cause-specific mortality differences across socioeconomic position of municipalities in Japan, 1973–1977 and 1993–1998: increased importance of injury and suicide in inequality for ages under 75. Int J Epidemiol 2005;34:100–9.
[36] Pickett KE, Kelly S, Brunner E, Lobstein T, Wilkinson RG. Wider income gaps, wider waistbands? An ecological study of obesity and income inequality. J Epidemiol Community Health 2005;59:670–4.
[37] Booth SL, Sallis JF, Ritenbaugh C, Hill JO, Birch LL, Frank LD, et al. Environmental and societal factors affect food choice and physical activity: rationale, influences, and leverage points. Nutr Rev 2001;59(3 pt 2):S21–39.
[38] Bjorntorp P, Rosmond R. Hypothalamic origin of the metabolic syndrome X. Ann N Y Acad Sci 1999;892:297–307.
[39] Chrousos GP. The role of stress and the hypothalamic–pituitary–adrenal axis in the pathogenesis of the metabolic syndrome: neuro-endocrine and target tissue-related causes. Int J Obes Relat Metab Disord 2000;24(suppl 2):S50–5.
[40] Ministry of Health, Labour and Welfare of Japan. The national health and nutrition survey in Japan, 2005 (in Japanese). Tokyo: Daiichi Shuppan Publishing Co Ltd; 2008.

Appendix

The members of the Japan Dietetic Students' Study for Nutrition and Biomarkers Group (in addition to the authors) are as follows: Mitsuyo Yamasaki, Yuko Hisatomi, Junko Soezima, and Kazumi Takedomi (Nishikyushu University); Toshiyuki Kohri and Naoko Kaba (Kinki University); Etsuko Uneoka (Otemae College of Nutrition); Hitomi Hayabuchi and Yoko Umeki (Fukuoka Women's University); Keiko Baba and Maiko Suzuki (Mie Chukyo University Junior College); Reiko Watanabe and Kanako Muramatsu (University of Niigata Prefecture); Kazuko Ohki, Seigo Shiga, Hidemichi Ebisawa, and Masako Fuwa (Showa Women's University); Tomoko Watanabe, Ayuho Suzuki, and Fumiyo Kudo (Chiba Prefectural University of Health Science); Katsumi Shibata, Tsutomu Fukuwatari, and Junko Hirose (The University of Shiga Prefecture); Toru Takahashi and Masako Kato (Mimasaka University); Toshinao Goda and Yoko Ichikawa (University of Shizuoka); Junko Suzuki, Yoko Niida, Satomi Morohashi, Chiaki Shimizu, and Naomi Takeuchi (Hokkaido Bunkyo University); Jun Oka and Tomoko Ide (Tokyo Kasei University); and Yoshiko Sugiyama and Mika Furuki (Minamikyushu University).

準体重群（BMI：18～24）では7～10kgとされている[8]．またHyttenらの報告によると健康初妊婦の妊娠時の平均体重増加量は12.5kgとしている[3]．米国のNational Academy of Scienceの医学部門における妊娠時の体重に関する勧告[9]では，やせには12.5～18kg，正常妊婦には11.5～16kg，肥満に対しては7～11.5kgが妊娠時の適正な体重増加量としている．ここでいうやせ・正常・肥満はbody mass index（BMI）でそれぞれ19.8未満，19.8～26，26以上で定義している．またBMIが29を超える場合は，少なくとも6kg以上の体重増加を必要としている．American College of Obstetricians and Gynecologists[10]もこのガイドラインを用いている．

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【参考文献】
[1] 日本肥満学会肥満症のてびき編集委員会編：肥満・肥満症の指導マニュアルpp. 1-26，医歯薬出版，1997.
[2] 厚生労働省：日本人の食事摂取基準（2005年版），第一出版，2005.
[3] Hytten FE, Leitch I.：The physiology of human pregnancy. 2nd edition. Blackwell Scientific Publications, Oxford.1971.
[4] FAO／WHO／UNU Expert Consultation：Report on human energy requirements, Interim Report, 2004.
[5] 井上五郎訳・必須アミノ酸研究委員会編：エネルギー・たんぱく質の必要量，FAO／WHO／UNU合同特別専門委員会，WHOテクニカル・レポート・シリーズ724，医歯薬出版，1994.
[6] Olsen SF, Secher NJ.：Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. BMJ 324:447-450, 2002.
[7] 一條元彦：栄養問題委員会報告 ― 婦人（非妊婦・妊婦）および胎児・新生児の体格現状調査（正常群），日本産科婦人科学会雑誌40：1487，1988.
[8] 研修ノートNo.64「妊娠中毒症」日本母性保護産婦人科医会，2001.
[9] Institute of Medicine: Subcommittee on nutritional status and weight gain in during pregnancy. Nutrition during pregnancy. Weight gain. National Academic Press, Washington DC, pp. 1-13, 190, 1990.
[10] American College of Obstetricians and Gynecologists: Nutrition during Pregnancy. Technical Bulletin No. 179, April 1993.

4.3　高齢者の栄養

栄養を考える上で重要な高齢者の特徴は，①加齢に伴う身体の機能および形態の変化，②個人差の大きさ，③疾病もしくは障害の保有率である．これらの特徴によって，高齢者では栄養の摂取や身体活動の低下，代謝の変化，疾病等による身体状況の悪化が見られ，一般に栄養障害を来たしやすい．高齢者の栄養状態・栄養必要量の評価に関しては，若年者よりも相応の注意を払うことが必要である．

1)　加齢による消化・吸収・代謝の変化

加齢により基礎代謝は低下するが，中高齢期での低下はそれ程著しくはなく，身体活動が活発な高齢者では加齢に伴う変化は非常に小さいという報告がある[1]．

一方，高齢者では委縮性胃炎のために胃酸分泌が減少している．そのため小腸において細菌の過増殖が起こり，小腸からの栄養素吸収が低下するという報告があり[2]，これが低栄養を引き起こす要因のひとつと考えられてきた．

炭水化物，脂質，たんぱく質の加齢による吸収率の低下は明らかではないが，炭水化物の代謝から見ると，加齢に伴いインスリン分泌が低下し，食後血糖値が上昇しやすくなる．また，骨格筋量が減少し，脂肪の割合が増加することにより，インスリン抵抗性の増大や骨格筋でのたんぱく代謝の低下が見られる．

他の栄養素では，胃酸および内因子の分泌減少により，ビタミンB_{12}の消化・吸収が低下すると報告されている[3]．カルシウムの腸管からの吸収率は若年者より低いことが知られてお

り，これは高齢者のビタミンDの栄養状態の低下や活性化ビタミンDの作用の低下などによる．

以上のように，さまざまな栄養素の消化・吸収・代謝が，加齢による影響を受ける可能性が指摘されている．しかしながら，一般に消化管の機能，形態は他の臓器ほどは加齢の影響を受けないものと考えられている．食道や胃の運動は高齢者では低下しているとされるが，胃の加齢変化と捉えられていた委縮性胃炎や胃酸分泌の低下は，近年，加齢に伴って増加する*Helicobacter pylori*感染によるものである可能性が示された[4]．少なくともヒトの小腸は形態的には加齢の影響をあまり受けないため[5]，栄養素の吸収に関しても，小腸の機能・形態の変化による影響を大きく受けることはないと考えられる．今のところ，加齢に伴う腸管からの栄養吸収障害が，高齢者の低栄養の主たる原因であるとの根拠はない．

2) 高齢者の栄養摂取量

高齢者の栄養摂取量を国民健康・栄養調査で見ると，エネルギーや脂質の摂取量は70歳未満の成人より平均的に下がっているが，他の栄養素に関しては，成人より摂取量が特に少ないという傾向は明らかではない(図3－3)．また，摂取量のばらつきが70歳以上で特に大きくなるという傾向も見られなかった．各栄養素の摂取量を日本人の食事摂取基準（2010年版）の高齢者（70歳以上）の基準値と比較した場合，カルシウム，亜鉛，ビタミンB_1などは，推定平均必要量未満しか摂取していない人の割合が男女とも50%を超えており，不足しやすい栄養素ということができる．

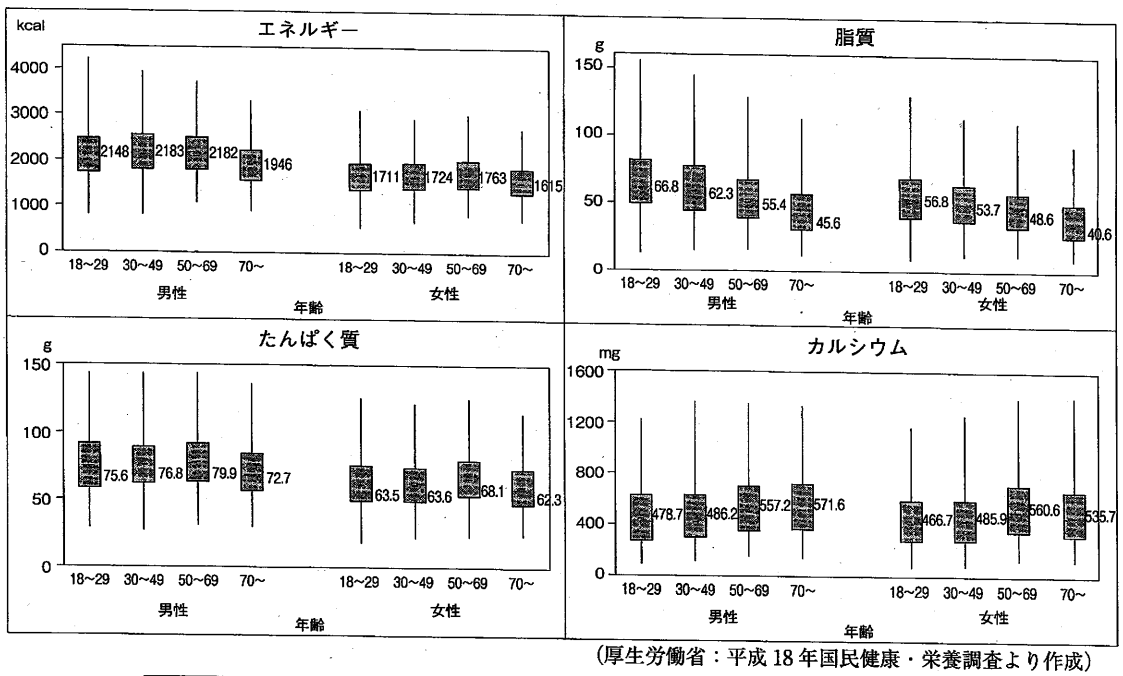
国民健康・栄養調査では，高齢者でも肥満(図3－4)，メタボリックシンドロームや糖尿病が高率に認められ，なおかつ増加傾向にある．調査の回答者は自立した健康な高齢者が主であると考えられ，このような自立高齢者では，過栄養が問題となってきていることが明らかである．上述したように，加齢に伴い筋肉や骨などの除脂肪体重（lean body mass）が減少し，脂肪組織の割合が増えるため，質的には肥満状態に向かいやすい．生活習慣病を予防するためには，青壮年期からの運動習慣による筋量・骨量の維持とともに，過不足のない適切な栄養摂取が重要である．

3) 有病者，要介護者の栄養状態

高齢者は何らかの疾患を有する率が高く，70歳以上では半数以上が病気やけが等で何らかの自覚症状がある（有訴者率＝520人/人口千人当たり）．また，70歳以上では人口の4.4%が入院していると推定される（入院受療率＝4,400人/人口10万人当たり）．介護保険の実施から見ると，65歳以上高齢者の16%が要介護(要支援)認定を受けており，介護サービスの利用者は350万人に上る．健康で自立した高齢者では個人差はそれほど大きくなく，低栄養状態もほとんど見られないが，高齢者全体で見ると，有病者や要介護者が大きな割合で存在している．施設居住者は，自立した高齢者よりも身体活動量や基礎代謝量が少なく[6]，経口から十分な栄養が摂取できずに低栄養状態にある人も少なくない．食事摂取量の不足による低栄養（protein-energy malnutrition：PEM）は，加齢に伴う筋肉量の減少と筋力の低下を加速し，sarcopeniaとよばれる状態を引き起こす．これは，高齢者の骨折や転倒，自立障害の大きな原因となっている．

4) 高齢者の栄養評価

高齢者，特に有病者や要介護者への適切な栄養対策を考える上では，栄養状態の正確な評価が必要不可欠である．栄養状態の評価は，①身体組成の測定，②血液データの測定，③食事摂



（厚生労働省：平成 18 年国民健康・栄養調査より作成）

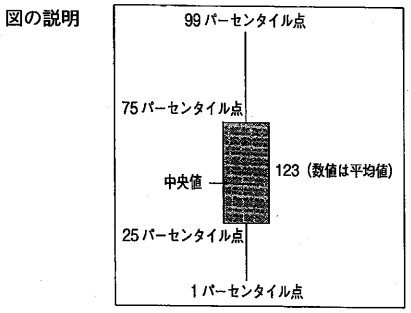
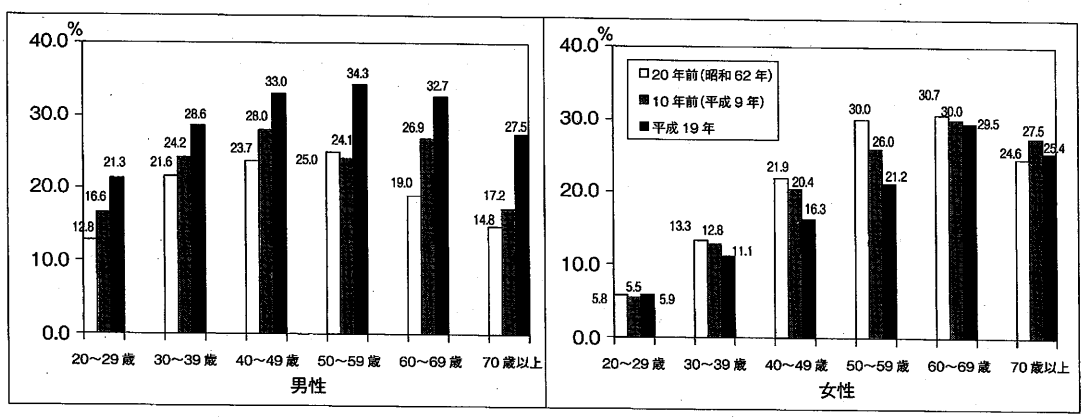


図３－３　年齢別栄養摂取量



（厚生労働省：平成 19 年国民健康・栄養調査概要より一部改変）

図３－４　年齢別肥満者（BMI ≧ 25）の割合

取量の評価，④身体活動の評価，⑤代謝状態の把握などからなる．

身体組成については，日本栄養アセスメント研究会が日本人の新身体計測基準値（JARD2001）を発表しており[7]，70～85歳までの5歳刻みおよび85歳以上の身長・体重や皮下脂肪厚等の基準値が算出されている．上腕の身体計測値などは，寝たままでも測定し比較することができるので，非常に有用である．ただしJARD2001策定のための調査対象者人数は，国民健康・栄養調査の約半分程度であるので，国民健康・栄養調査から求められている食事摂取基準（2010年版）の基準体位なども参考にするのがよい．身体組成や血液データは，ある一時点での結果ではなく，一定期間に指標がどの程度変化したかを評価することが重要である．例えば体重で，1か月に5%以上，1週間に2%以上の減少などが観察された場合は，値にかかわらず要注意である．

食事摂取量は，高齢者本人やその家族への聞き取り調査や，入院入所者では給与食事量と残食量などから，栄養計算によって把握する．摂取量の変化や，摂取状況－咀嚼・嚥下困難，食欲（拒食，過食），食物の認知－などに留意する必要がある．

身体活動の評価は，基本的な日常生活動作（(basic) activity of daily living：ADL），手段的日常生活動作（instrumental ADL）など身体機能の評価をはじめとして，ある程度自立した人の場合には，歩数の測定や運動・生活活動の聞き取りによって把握することができる．障害や疾病を有している場合は，代謝性ストレスによる消費エネルギーの増加，消化・吸収・代謝機能の変化，薬剤の服用による影響など，栄養に関連する代謝状態の把握も非常に重要である．

栄養状態の評価ツールとしては，高齢者用にMini Nutritional Assessment：MNAが開発されており，日本人でもその妥当性が検証されている[8]．初めに6項目からなるスクリーニングでリスクの高い者を割り出し，残り12項目で低栄養状態の評価を行うようになっている．計測項目も少なく簡便な評価ツールのひとつである．

5) 高齢者の栄養指導・栄養療法

高齢者への栄養指導・栄養療法を行うにあたっては，まず正確な栄養状態の評価を行い，対象者に必要でなおかつ摂取可能な栄養（食事）の提案および提供を行わなければならない．

自立高齢者については，食事摂取基準（2010年版）の値を参考にして，対象者の健康状態，身体活動量，生活環境なども考慮した上で，エネルギーなど必要な栄養量を算出する．高齢者で一般的に不足しやすい栄養素に注意するとともに，軽微な疾患を持つ場合には服薬の影響や代謝特性も念頭に入れて，対象者の食生活を考慮し，過剰や不足の栄養素がないよう計画を立てて指導することが必要である．

要介護・要医療の高齢者については，「不足」を中心とする栄養障害の予防・改善が，基礎疾患の治療，合併症の予防，生命予後などに大きく影響を及ぼす．従って，健常高齢者と同様に，正確な栄養評価を行い必要な栄養量を算出することはもちろんだが，栄養提供方法も主要な検討事項となる．経口摂取が基本ではあるが，必要に応じて経腸栄養や経静脈栄養を適切に実施し，特別用途食品（総合栄養食品やえん下困難者用食品）なども利用して，効率的に早期に栄養状態を改善することが，非常に重要である．

［森田明美］

【参考文献】
[1] L. A. Murray, J. J. Reilly, M. Choudhry, J. V. Durnin: A longitudinal study of changes in body composition and basal metabolism in physically

active elderly men. Eur J Appl Physiol Occup Physiol., 72(3), 215-218, 1996.
[2] A. Parlesak, B. Klein, K. Schecher, J. C. Bode, C. Bode: Prevalence of small bowel bacterial overgrowth and its association with nutrition intake in nonhospitalized older adults. J Am Geriatr Soc., 51(6), 768-773, 2003.
[3] J. D. Scarlett, H. Read, K. O'Dea: Protein-bound cobalamin absorption declines in the elderly. Am J Hematol., 39, 79-83, 1992.
[4] K. Haruma, T. Kamada, H. Kawaguchi, S. Okamoto, et al.: Effect of age and Helicobacter pylori infection on gastric acid secretion. J Gastroenterol Hepatol., 15, 277-283, 2000.
[5] G.R.Corazza, M. Frazzoni, M. R. Gatto, G. Gasbarrini: Ageing and small-bowel mucosa: a morphometric study. Gerontology., 32, 60-65, 1986.
[6] 横関利子：「高齢者の基礎代謝量と身体活動量」日本栄養・食糧学会誌. 46, 451-458, 1993.
[7] 細谷憲政, 岡田正, 武藤泰敏, 山森秀夫, 田代亜彦, 三輪佳行ほか：「日本人の新身体計測基準値（JARD2001）」栄養－評価と治療. 19, S46-81, 2002.
[8] Kuzuya M, Kanda S, Koike T, Suzuki Y, Satake S, Iguchi A：Evaluation of Mini-Nutritional Assessment for Japanese frail elderly. Nutrition., 21(4), 498-503, 2005.

5. 食　育

5.1 食育基本法と食育推進基本計画

今日，栄養の過剰や不足，不規則な食生活による生活習慣病の増加，家族との共食の減少，健康食品やサプリメントへの依存，食の安全・安心の揺らぎ，依然として先進国中最低の水準にある食料自給率など，食生活をめぐって多くの課題がある．こうした課題解決に向けて，2007年6月食育基本法が制定され，翌7月から施行された．個人の食生活は，どのような食物が入手可能かといった地域社会のフードシステムや，健康・食情報の提供など，食環境の影響により規定される部分が少なくない．したがって，「食」を単に個人の問題としてだけ考えていては課題解決に至らない場合が多い．そこで，社会として「食」のあるべき方向を議論し，取り組む必要性が生じてきた．

「食育」ということばは，明治時代の軍医である石塚左玄が記した書物で使われたのが最初とされる．食育基本法の前文の中では，「食育」は以下のとおり，人間形成にとって最も基本的なこととして位置づけられた．

食育基本法　前文より
子どもたちが豊かな人間性をはぐくみ，生きる力を身につけていくためには，何よりも「食」が重要である．今，改めて，食育を，生きる上での基本であって，知育，徳育及び体育の基礎となるべきものと位置付けるとともに，さまざまな体験を通じて「食」に関する知識と「食」を選択する力を習得し，健全な食生活を実践することができる人間を育てる食育を推進することが求められている．

また，食育の具体的な内容として，以下の点が挙げられている．

- 国民の心身の健康と豊かな人間性をはぐくむ（第2条）
- 食に関する感謝の念と理解をはぐくむ（第3条）
- 食育推進運動の展開：自発性の尊重，地域特性への配慮，主体的参加（第4条）
- 子どもの食育推進における保護者（家庭），教育関係者等の役割（第5条）
- 食料の生産から消費に至るまでの食に関するさまざまな体験活動の重視（第6条）
- 伝統的な食文化，環境と調和した生産等への配慮，農産漁村の活性化，食料自給率の向上への貢献（第7条）
- 食品の安全性の確保，食の安心（第8条）

また，平成18年3月には，内閣総理大臣を会長とする食育推進会議において食育推進基本計画が策定され，食育推進の基本方針や，食育としてめざす数値目標が示された．表3－11は9つの目標項目に関する策定時の数値，平成21年の現状値，平成22年度迄に達成をめざす目標値である[1]．このような数値目標を食育に

Original Article

Intake of dairy products and bone ultrasound measurement in late adolescents: a nationwide cross-sectional study in Japan

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Introduction: There is little evidence regarding the effects of dairy product intake on bone mineralization among late adolescents, especially in Asians. The aim of this study was to determine the association between dairy product intake and bone strength as measured by quantitative ultrasound (QUS) in a large Japanese population. Methods: Subjects were 38,719 high school students (14,996 males and 23,723 females) across 33 prefectures in Japan. Bone stiffness of the calcaneus was measured by QUS densitometry (AOS-100, Aloka). Subjects were given a self-administered questionnaire, which included questions on gender, age, height, weight, consumption of dairy products, and levels of physical activity. Intake of milk and yogurt were classified as none, 1-99, 100-199, 200-399, and ≥400 ml/day. Results: The proportion of subjects who consumed milk 400 ml/day or more was 21% in males and 7.3% in females, while 24% of males and 41.1% of females did not consume milk. After adjusting for physical activity, weight, gender, age, and area of residence, milk intake (R^2=2.8%, p<0.001) and yogurt intake (R^2=0.1%, p<0.001) were independently associated with the QUS measurement. Similar associations were found in males and females when a gender-stratified analysis was conducted. Conclusion: We found a positive dose-effect relationship between milk intake and bone strength in late adolescents, to whom we recommend milk intake of 400 ml/day or more to obtain greater bone mass.

Key Words: adolescent, bone density, calcium, dairy products, quantitative ultrasound measurement

INTRODUCTION

Dairy products, especially milk from cows, are an important source of calcium (Ca), which is essential for maintaining normal skeletal growth in children and adolescents. Many observational and interventional studies have shown that adequate Ca intake is required to attain maximal peak bone mass.[1,2] In addition, several researchers have demonstrated a favorable effect of increased milk and other dairy intake on bone parameters.[3-8] However, Lanou et al.[9] conducted a systematic review that revealed insufficient evidence to support the idea that increased intake of milk or other dairy products has a favorable effect on promoting bone mineralization in children and adolescents. They also pointed out that such evidence is scarce in non-Caucasian children and adolescents.

Dairy products are not popular in Japan. However, almost all elementary schools and most junior high schools provide students with a bottle of milk (200 ml) as part of the school lunches, which is supervised by a registered dietician. This enables students to maintain a relatively high Ca intake. The median Ca intake of students in the age groups 6-8, 9-11, and 12-14 years old were 614, 706, and 788 mg/day in males and 586, 659, and 656 mg/day for females, respectively.[10] However, high school students are rarely provided with school lunch (i.e., milk) and, therefore, their Ca intake is lower. The median Ca intake of students 15-17 years old was 570 mg/day in males and 467 mg/day for females.[10]

Despite ample evidence regarding the positive effect of increased Ca intake on bone mass in pre- and peripubertal children, less is known about the influence of Ca intake on bone mineral accretion in the late teenage years.[11] We hypothesized that increased Ca intake from dairy products is important for Japanese high school students whose Ca intake is low. The aim of this study was to determine the association between intake of dairy products and bone strength, as measured by quantitative ultrasound (QUS) in a large population of late Japanese adolescents.

MATERIALS AND METHODS

Subjects

We targeted high school students aged 15-18 years. We

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contacted 33 prefectures (of 47 prefectures) in Japan and invited high schools to join our study. In total, 236 high schools participated on a voluntary basis. Among the targeted 40,111 students, 38,719 students (96.5%, 14,996 males and 23,723 females) agreed to participate in this study. This study was conducted according to the guidelines established by the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee of Kagawa Nutrition University. Written informed consent was obtained from all subjects. QUS measurements and surveys using self-administered questionnaires were conducted in each high school from summer of 2006 to fall of 2007.

QUS Measurement

The strength of the calcaneus, or heel bone, of the right foot was evaluated with QUS densitometry using AOS-100 (Aloka, Tokyo). A QUS device measures elasticity and bone strength, which is dependent on both bone mass and bone architecture. The AOS-100 measures the speed of sound (SOS; in m/second) and the transmission index (TI). TI values are related to frequency-dependent attenuation. SOS and TI values measured with the AOS-100 are highly correlated with the SOS (r=0.89) and broadband ultrasound attenuation (BUA) (r=0.88) measured with a conventional QUS device (UBA575+, Hologic Inc.).[12] Osteo sono-assessment index (OSI), which is an estimate of elastic modules equal to the product of physical density and the square of SOS, was calculated as a combined parameter of SOS and TI by multiplying TI by the square of SOS.[12] OSI showed better reproducibility than BUA. The validity of the present QUS measurement has been previously published and coefficients of variation of SOS, TI, and OSI measurements were 0.3%, 1.2%, and 1.6%, respectively, [12] which is within the standard value provided by the manufacturer.

Demographic and Lifestyle Characteristics

The self-administered questionnaire included questions regarding the subject's gender, age, height, weight, current consumption of dairy products, and current physical activity level. Subjects reported their height and weight to the nearest 1 cm and 1 kg, respectively, and body mass index (BMI) was calculated by dividing weight (kg) by the square of height (m^2). Intake of milk and yogurt was classified as none, 1-99 ml/day, 100-199 ml/day, 200-399 ml/day, and ≥400 ml/day; the intake of cheese was classified as none, 1-19 g/day, 20-39 g/day, 40-59 g/day, and

Table 1. Subject characteristics

	Mean or Proportion		
	Male (n=14,996)	Female (n=23,723)	*p* value
Age (years)	16.3 (SD 0.9)	16.4 (SD 0.9)	<0.001
Height (cm)	170.2 (SD 5.9)	157.8 (SD 5.4)	<0.001
Weight (kg)	61.4 (SD 10.6)	51.5 (SD 7.7)	<0.001
Body mass index (kg/m^2)	21.2 (SD 3.2)	20.7 (SD 2.8)	<0.001
Speed of sound (m/sec)	1577 (SD 174)	1592 (SD 164)	<0.001
Osteo sono-assessment index (10^6)	3.06 (SD 0.40)	2.88 (SD 0.35)	<0.001
Milk intake (ml/day)			
0	24.0%	41.1%	<0.001
>0 to <100	14.4%	19.1%	χ^2=2597
≥100 to <200	14.8%	15.4%	
≥200 to <400	25.8%	17.2%	
≥400	21.0%	7.3%	
Yogurt intake (ml/day)			
0	43.0%	37.7%	<0.001
>0 to <100	35.0%	38.7%	χ^2=193
≥100 to <200	14.2%	17.2%	
≥200 to <400	5.8%	5.0%	
≥400	2.0%	1.3%	
Cheese intake (g/day)			
0	62.0%	61.8%	<0.001
>0 to <20	19.9%	21.3%	χ^2=78
≥20 to <40	9.6%	10.4%	
≥40 to <60	4.1%	3.5%	
≥60	4.4%	3.0%	
Frequency of exercise (times/week)			
≤1	36.1%	61.4%	<0.001
2- 3	10.6%	11.3%	χ^2=2836
4- 5	10.3%	6.3%	
≥6	43.0%	21.0%	

≥60 g/day. These classifications are easy to understand for most Japanese because a bottle or a pack of milk has historically corresponded to approximately 200 ml, a cup of yogurt has corresponded to approximately 100 ml, and a standard serving size of cheese corresponds to 20 g. As for physical activity, subjects were asked to report the frequency of their current, weekly exercise regime.

Statistical analysis

All continuous variables were checked for normality. Weight and BMI were skewed to higher values, and were transformed logarithmically prior to conducting statistical tests. Student's *t*-test was used to test for differences between two groups. The chi-square test was used to test for independence of categorical data. Regression analyses were used to assess the linear association between predictor variables and the QUS value. In multiple regression analyses, exercise habit, body statue, gender, age, and area of residence (either in Tohoku, Kanto, Chubu, Kinki, Chugoku, Shikoku, or Kyushu area in Japan) were considered as covariates. The parameter "area" was treated as a dummy variable. SAS statistical package (version 9.13, SAS Institute, Cary, NC, USA) was used for all analyses. A *p*-value <0.05 was considered significant.

RESULTS

Table 1 shows the characteristics of all subjects. Mean values of age, height, weight, BMI, and OSI were significantly different between males and females. Males had higher milk intake and exercised more frequently than females. There was a slight but significant difference in yogurt and cheese intake between males and females.

Table 2 shows the results of simple linear regression analyses with OSI as the outcome. All predictor variables were significantly associated with OSI. Weight was the strongest predictor of OSI, followed by exercise frequency, BMI, gender, milk intake, and age. Table 2 also shows results according to gender. Figure 1 illustrates mean OSI by levels of milk intake. The mean OSI of the milk intake groups were higher than the no milk intake group, by 0.7% in the 1-99ml-group, 2% in the 100-199ml-group, 3.8% in the 200-399ml-group, and 6.7% in the ≥400ml-group. Figure 2 illustrates mean OSI by levels of milk intake according to gender. The pattern of association between OSI and milk intake was similar between genders.

Table 2. Simple linear regression analyses with OSI as the dependent variable by gender

Predictor variable	Regression coefficient (b)	R^2 (%)	*p*-value
Total			
Milk intake*	0.044	2.8	<0.001
Yogurt intake*	0.025	0.4	<0.001
Cheese intake†	0.01	0.1	<0.001
Frequency of exercise‡	0.075	6.8	<0.001
Weight (kg)§	0.746	11.2	<0.001
Body mass index (kg/m^2)§	0.709	6	<0.001
Gender (1, male; 0, female)	0.184	5.6	<0.001
Age (years)	0.043	1.1	<0.001
Males			
Milk intake*	0.034	1.6	<0.001
Yogurt intake*	0.028	0.5	<0.001
Cheese intake†	0.009	0.1	0.003
Frequency of exercise‡	0.048	2.7	<0.001
Weight (kg)§	0.587	5.6	<0.001
Body mass index (kg/m^2)§	0.64	5.1	<0.001
Age (years)	0.074	3.1	<0.001
Females			
Milk intake*	0.027	1.1	<0.001
Yogurt intake*	0.026	0.5	<0.001
Cheese intake†	0.008	0.1	<0.001
Frequency of exercise‡	0.071	6.2	<0.001
Weight (kg)§	0.689	7.6	<0.001
Body mass index (kg/m^2)§	0.676	5.8	<0.001
Age (years)	0.029	0.6	<0.001

*Coded as 1: 0 ml/day; 2: >0 to <100 ml/day; 3: ≥100 to <200 ml/day; 4: ≥200 to <400 ml/day; 5: ≥400 ml/day
†Coded as 1: 0 g/day; 2: >0 to <20 g/day; 3: ≥20 to <40 g/day; 4: ≥40 to <60 g/day; 5: ≥60 g/day
‡Coded as 1: ≤1 time/week; 2: 2-3 times/week; 3: 4-5 times/week; 4: ≥6 times/week
§Logarithmically transformed

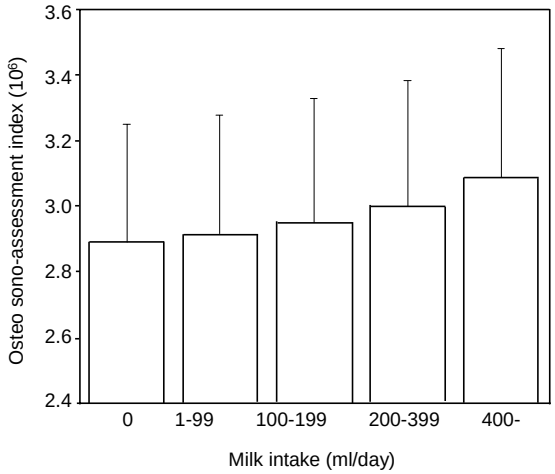

Figure 1. Mean osteo sono-assessment index (OSI) by levels of milk intake. Mean OSIs in milk intake groups were higher than the no milk intake group.

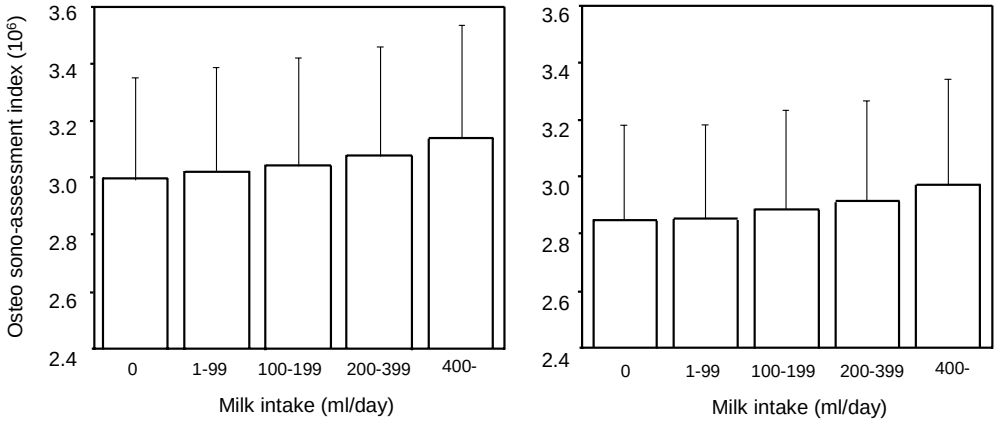

Figure 2. Mean osteo sono-assessment index (OSI) by levels of milk intake in males (left) and females (right). The pattern of association between OSI and milk intake was similar between genders, with R^2 vales of milk intake being 1.6 % for males and 1.1% for females.

Table 3 shows the results of multiple regression analyses. In this multivariate model, we included weight as the index of body statue, but did not include BMI because BMI was highly correlated with weight (r=0.821). After adjusting for exercise frequency, weight, gender, age, and area of residence, regression analyses indicated that milk and yogurt intake were independently associated with OSI, while cheese intake was not. The interaction of milk intake and exercise frequency was not significant (p=0.424) when the interaction term was added to the multivariate model. Table 3 shows these results according to gender. R^2 values of milk intake in males (1.6%) and females (1.1%) were lower than that in males and females combined (2.8%).

DISCUSSION

The relationship between Ca intake and bone health in children and adolescents is an area of interest for clinicians and epidemiologists. Most recently, Winzenberg *et al*.[13] demonstrated in their meta-analysis of randomized clinical trials that Ca supplementation has a small effect on the bone mineral density (BMD) of upper arm limbs and total body, but no effect on hip or vertebral BMD. Their study, however, has several limitations. The baseline Ca intake in most studies enrolled in the meta-analysis ranged from moderate to high. Another meta-analysis has revealed that Ca supplementation among subjects with low Ca intake has a positive effect on bone mineral content (BMC), much larger than that seen among children with normal Ca intake.[14] Therefore, it is important to demonstrate this association in low-Ca-intake populations. The present study did not evaluate total Ca intake. However, the results of the National Health and Nutrition Survey can be applied to the present study because we used a nationwide sample. The 2004 National Health and Nutrition Survey reported that the median Ca intake among 15-17 year old adolescents was 570 mg/day in males and 467 mg/day in females, which are both lower than the recommended dietary reference intakes for Japanese of 1100 mg/day and 850 mg/day, respectively.[15] The subjects in the present study represent a low Ca intake population.

The present study demonstrated a clear dose-effect relationship between milk intake and calcaneal QUS values in adolescents aged 15-18 years, thereby demonstrating the importance of milk for bone health in Japanese adolescents. Specifically, milk intake of 400 ml/day or more is recommended to attain maximal peak bone mass. Sev-

Table 3. Multiple linear regression analyses with OSI as the dependent variable by gender

Predictor variable*	Regression coefficient (b)	R^2 (%)	*p*-value
Total	Overall R^2=20.0		
Milk intake†	0.016	2.8	<0.001
Yogurt intake†	0.011	0.1	<0.001
Cheese intake‡	0.00005	0	0.978
Frequency of exercise§	0.064	5.3	<0.001
Weight (kg)¶	0.599	8.1	<0.001
Gender (1, male; 0, female)	0.035	0.1	<0.001
Age (years)	0.052	1.7	<0.001
Males	Overall R^2=15.2		
Milk intake†	0.02	1.6	<0.001
Yogurt intake†	0.01	0.2	0.002
Cheese intake‡	-0.003	0	0.372
Frequency of exercise§	0.059	1.9	<0.001
Weight (kg)¶	0.512	5.1	<0.001
Age (years)	0.082	3.7	<0.001
Females	Overall R^2=16.4		
Milk intake†	0.013	1.1	<0.001
Yogurt intake†	0.012	0.2	<0.001
Cheese intake‡	0.002	0	0.453
Frequency of exercise§	0.07	5.5	<0.001
Weight (kg)¶	0.659	7	<0.001
Age (years)	0.033	0.9	<0.001

*Dummy variable of "area of residence" is also included in the multivariate model in addition to the variables shown in this table
†Coded as 1: 0 ml/day; 2: >0 to <100 ml/day; 3: ≥100 to <200 ml/day; 4: ≥200 to <400 ml/day; 5: ≥400 ml/day
‡Coded as 1: 0 g/day; 2: >0 to <20 g/day; 3: ≥20 to <40 g/day; 4: ≥40 to, <60 g/day; 5: ≥60 g/day
§Coded as 1: ≤1 time/week; 2: 2-3 times/week; 3: 4-5 times/week; 4: ≥6 times/week
¶Logarithmically transformed

eral studies have tried to determine the association between milk intake and bone mass in adolescents. In one study, dairy food supplements, including 300-400 mg/day of Ca, increased the bone mass of various bone sites among Caucasian adolescents.[3,5] Matkovic *et al*.[16] showed that Ca and dairy products influence bone mass acquisition in 15-18 year old females, leading to a higher peak bone mass. They also determined that Ca exerts its action on bone accretion during growth, primarily by influencing volumetric bone mineral density, while dairy products may have an additional impact on bone growth and periosteal bone expansion. One study has specifically been conducted among East Asians, and showed that the high-milk intake group (128 mg/day of milk) had significantly higher BMC at the distal radius than the no-milk intake group of Chinese girls aged 12-14 years.[6] These previous studies are all in line with the results of our study.

Studies conducted on the association between Ca intake and bone mass in late teens are much fewer than in pre- and peripubertal children.[11,13,14] Two interventional studies targeting late adolescents showed that Ca supplementation was effective for bone mass accrual.[17,18] An observational study using a QUS device showed that Ca intake is associated with calcaneal QUS values in American girls aged 14-18 years.[19] All of these studies, including the present study, suggest that a favorable effect of Ca and dairy foods on bone mass or bone growth is consistently observed in late adolescents.

Yogurt intake was also associated with QUS values independently, but its contribution was small (R^2=0.1%), while cheese intake was not associated with QUS. This finding can be explained partly by the fact that intake of yogurt and cheese was less than that of milk. In general, dairy products do not appear to be common foods in the dietary habits of the Japanese. Yogurt was not consumed by 40% of the subjects in this study, and cheese was not consumed by 60%. Furthermore, milk was not consumed in 40% of female subjects. The National Health and Nutrition Survey demonstrated that dairy products only account for 30% of total Ca intake.[10] Low Ca intake due to low dairy product intake is common in Asians.[6,20] Therefore, increased intake of dairy products may be an effective strategy to maximize peak bone mass in late Asian adolescents.

The prevalence of lactose intolerance in the Japanese, like that in other Eastern Asians,[6] is high; the prevalence in Japanese high school students is reported to be 33.8%.[21] As the relatively lower consumption of dairy products in Japanese girls may be due in part to the symptoms of lactose intolerance, strategies other than simply encouraging

the consumption of dairy products may be needed to increase their calcium intake.

Physical activity has been shown to be another key factor associated with bone mass in late adolescents.[19,22,23] In the present study, physical activity was found to be a more influential factor on QUS values than milk intake, especially for females. This finding was partly explained by the fact that many subjects exercised frequently; 43% of males and 21% of females reported exercising six or more times a week. Many high school students in Japan enthusiastically participate in school sports club activities, and thus both the frequency and intensity of their physical activity is considered to be high.

This study has many strengths. The sample size of this study was large, such that we had sufficient statistical power to detect a possible association between dairy product intake and QUS values. Furthermore, subjects were recruited from various regions of Japan, which makes the results generalizable to all Japanese late adolescents. This study also has several limitations. First, we measured bone stiffness of only the calcaneus using a QUS device. Although this technique is useful in estimating bone mass or bone growth in adolescents,[19,24,25] we did not obtain information on other bone sites of interest, such as the proximal femur and vertebrae. The effect of dairy products on these bones may not be the same as that seen with the calcaneus, and thus this needs to be clarified in future studies. Second, we did not evaluate pubertal status, which would have had an effect on bone status, especially for younger subjects (only a few years after puberty onset). Third, information on consumption of dairy products was self-reported. One may drink milk by family- or economic-packs rather than from individual packages or bottles. This method of evaluation is associated with potential measurement bias, which may have attenuated the association between milk intake and QUS values. Finally, the design of this study was cross-sectional, which does not necessarily indicate causal relationships. Intervention studies are needed to remedy these problems.

The present study demonstrated a dose-effect relationship between milk intake and bone strength in late adolescents, to whom we recommend an intake of 400 ml/day or more to obtain greater bone mass. These findings may be generalizable to populations whose dairy product intake is generally low.

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AUTHOR DISCLOSURES

The authors have no conflicts of interest.

REFERENCES

1. Anderson JJ. Calcium requirements during adolescence to maximize bone health. J Am Coll Nutr. 2001;20(S2):186S-91S.
2. Heaney RP, Weaver CM. Newer perspectives on calcium nutrition and bone quality. J Am Coll Nutr. 2005;24(S6): 574S-81S.
3. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial. BMJ. 1997;315:1255-60.
4. Teegarden D, Lyle RM, Proulx WR, Johnston CC, Weaver CM. Previous milk consumption is associated with greater bone density in young women. Am J Clin Nutr. 1999;69: 1014-7.
5. Merrilees MJ, Smart EJ, Gilchrist NL, Frampton C, Turner JG, Hooke E, March RL, Maguire P. Effects of diary food supplements on bone mineral density in teenage girls. Eur J Nutr. 2000;39:256-62.
6. Du XQ, Greenfield H, Fraser DR, Ge KY, Liu ZH, He W. Milk consumption and bone mineral content in Chinese adolescent girls. Bone. 2002;30:521-8.
7. Novotny R, Daida YG, Grove JS, Acharya S, Vogt TM, Paperny D. Adolescent dairy consumption and physical activity associated with bone mass. Prev Med. 2004;39:355-60.
8. Huncharek M, Muscat J, Kupelnick B. Impact of dairy products and dietary calcium on bone-mineral content in children: results of a meta-analysis. Bone. 2008;43:312-21.
9. Lanou AJ, Berkow SE, Barnard ND. Calcium, dairy products, and bone health in children and young adults: a re-evaluation of the evidence. Pediatrics. 2005;115:736-43.
10. Society for Information on Health and Nutrition. The National Health and Nutrition Survey in Japan, 2004. Daiichi Shuppan, Tokyo; 2006.Japanese.
11. Prentice A, Ginty F, Stear SJ, Jones SC, Laskey MA, Cole TJ. Calcium supplementation increases stature and bone mineral mass of 16- to 18-year-old boys. J Clin Endocrinol Metab. 2005;90:3153-61.
12. Tsuda-Futami E, Hans D, Njeh CF, Fuerst T, Fan B, Li J, He YQ, Genant HK. An evaluation of a new gel-coupled ultrasound device for the quantitative assessment of bone. Br J Radiol. 1999;72:691-700.
13. Winzenberg T, Shaw K, Fryer J, Jones G. Effects of calcium supplementation on bone density in healthy children: meta-analysis of randomised controlled trials. BMJ. 2006;333:775.
14. Huncharek M, Muscat J, Kupelnick B. Impact of dairy products and dietary calcium on bone-mineral content in children: results of a meta-analysis. Bone. 2008;43:312-21.
15. Ministry of Health, Labour, and Welfare, Japan. Dietary Reference Intakes for Japanese, 2005. Ministry of Health, Labour, and Welfare, Japan, Tokyo; 2004. (In Japanese).
16. Matkovic V, Landoll JD, Badenhop-Stevens NE, Ha EY, Crncevic-Orlic Z, Li B, Goel P. Nutrition influences skeletal development from childhood to adulthood: a study of hip, spine, and forearm in adolescent females. J Nutr. 2004;134: 701S-5S.
17. Stear SJ, Prentice A, Jones SC, Cole TJ. Effect of a calcium and exercise intervention on the bone mineral status of 16-18-y-old adolescent girls. Am J Clin Nutr. 2003;77:985-92.
18. Ho SC, Guldan GS, Woo J, Yu R, Tse MM, Sham A, Cheng J. A prospective study of the effects of 1-year calcium-fortified soy milk supplementation on dietary calcium intake and bone health in Chinese adolescent girls aged 14 to 16. Osteoporos Int. 2005;16:1907-16.
19. Robinson ML, Winters-Stone K, Gabel K, Dolny D. Modifiable lifestyle factors affecting bone health using calcaneus quantitative ultrasound in adolescent girls. Osteoporos Int. 2007;18:1101-7.
20. Novotny R, Boushey C, Bock MA, Peck L, Auld G, Bruhn CM et al. Calcium intake of Asian, Hispanic and white youth. J Am Coll Nutr. 2003;22:64-70.

21. Kosuge N, Yoshimatsu M, Tsukada K. Investigation into lactose absorption in Japanese children and adults: relation to intake of milk and dairy products. J Jpn Pediatr Soc. 1998;102:1090-7. (in Japanese)
22. French SA, Fulkerson JA, Story M. Increasing weight-bearing physical activity and calcium intake for bone mass growth in children and adolescents: a review of intervention trials. Prev Med. 2000;31:722-31.
23. Foo LH, Zhang Q, Zhu K, Ma G, Greenfield H, Fraser DR. Influence of body composition, muscle strength, diet and physical activity on total body and forearm bone mass in Chinese adolescent girls. Br J Nutr. 2007;98:1281-7.
24. Cvijetić S, Barić IC, Bolanca S, Juresa V, Ozegović DD. Ultrasound bone measurement in children and adolescents. Correlation with nutrition, puberty, anthropometry, and physical activity. J Clin Epidemiol. 2003;56:591-7.
25. Babaroutsi E, Magkos F, Manios Y, Sidossis LS. Lifestyle factors affecting heel ultrasound in Greek females across different life stages. Osteoporos Int. 2005;16:552-61.

Original Article

Intake of dairy products and bone ultrasound measurement in late adolescents: a nationwide cross-sectional study in Japan

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乳製品攝取與青少年晚期骨骼超音波測量：日本全國橫斷性研究

前言：目前很少，特別是對亞洲人，關於乳製品的攝取與青少年晚期骨骼礦化影響的實證資料。本篇研究之目的在於評估日本大型族群乳製品的攝取與定量超音波儀(QUS)測量之骨骼強度間的關係。方法：共有遍及 33 縣的 38,719 位高級中學的學生為個案(14,996 位男生及 23,723 位女生)。跟骨硬度以定量超音波儀(AOS-100，Aloka)測量。給予個案一份自評問卷，包含性別、年齡、身高、體重、乳製品攝取及體能活動情形。鮮奶及酸奶的攝取被分類為無攝取、每天攝取 1-99、100-199、200-399 及≥400 mL。結果：鮮奶每天攝取≥400 mL 的比例在男生為 21%，女生為 7.3%；另外有 24%的男生及 41.1%的女生無攝取鮮奶。在校正體能活動、體重、性別、年齡及居住區域後，QUS 測量值與鮮奶攝取(R^2=2.8%，p<0.001)及酸奶攝取(R^2=0.1%，p<0.001)分別有相關。將男生與女生按性別分層後，仍有類似的結果。結論：青少年晚期的鮮奶攝取量與骨骼強度之間呈現正向的劑量效應，因此我們建議，此段年齡的青少年每天至少攝取鮮奶 400 mL，以獲得較好的骨質。

關鍵字：青少年、骨密度、鈣、乳製品、定量超音波測量

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Article

Fractional Absorption of Active Absorbable Algal Calcium (AAACa) and Calcium Carbonate Measured by a Dual Stable-Isotope Method

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Abstract: With the use of stable isotopes, this study aimed to compare the bioavailability of active absorbable algal calcium (AAACa), obtained from oyster shell powder heated to a high temperature, with an additional heated seaweed component (Heated Algal Ingredient,

HAI), with that of calcium carbonate. In 10 postmenopausal women volunteers aged 59 to 77 years (mean ± S.D., 67 ± 5.3), the fractional calcium absorption of AAACa and $CaCO_3$ was measured by a dual stable isotope method. ^{44}Ca-enriched $CaCO_3$ and AAACa were administered in all subjects one month apart. After a fixed-menu breakfast and pre-test urine collection (Urine 0), ^{42}Ca-enriched $CaCl_2$ was intravenously injected, followed by oral administration of ^{44}Ca-enriched $CaCO_3$ without carrier 15 minutes later, and complete urine collection for the next 24 hours (Urine 24). The fractional calcium absorption was calculated as the ratio of Augmentation of ^{44}Ca from Urine 0 to Urine 24/ augmentation of ^{42}Ca from Urine 0 to Urine 24. Differences and changes of ^{44}Ca and ^{42}Ca were corrected by comparing each with ^{43}Ca. Fractional absorption of AAACa (mean ± S.D., 23.1 ± 6.4), was distinctly and significantly higher than that of $CaCO_3$ (14.7 ± 6.4; $p = 0.0060$ by paired t-test). The mean fractional absorption was approximately 1.57-times higher for AAACa than for $CaCO_3$. The serum 25(OH) vitamin D level was low (mean ± S.D., 14.2 ± 4.95 ng/ml), as is common in this age group in Japan. Among the parameters of the bone and mineral metabolism measured, none displayed a significant correlation with the fractional absorption of $CaCO_3$ and AAACa. Higher fractional absorption of AAACa compared with $CaCO_3$ supports previous reports on the more beneficial effect of AAACa than $CaCO_3$ for osteoporosis.

Keywords: active absorbable algal calcium (AAACa); calcium carbonate; dual stable Ca isotope method; fractional absorption (FA); parathyroid hormone (PTH)

1. Introduction

Active absorbable algal calcium (AAACa) prepared from heated oyster shell and seaweed is a unique calcium supplement with high bioavailability, with a characteristic lamellar crystalline structure quite unlike that of calcium oxide and calcium carbonate ($CaCO_3$) [1]. In the Katsuragi Calcium study, a prospective, randomized, double blind and placebo-controlled study compared the effect of AAACa on osteoporosis with that of $CaCO_3$ in hospitalized women with a mean age of 80 years. It was found that AAACa alone increased spinal bone mineral density significantly over the level in subjects given a placebo, whereas $CaCO_3$ did not [2,3]. Fracture occurrence over the two year test period from among 58 subjects was 0 of 5 in the AAACa Group, 2 of 7 in the $CaCO_3$ Group and 3 of 5 in the Placebo Group, on evaluation of all X-rays available at the beginning and end of the test period. The AAACa Group exhibited a significantly lower rate of fracture occurrence than the placebo group, but the $CaCO_3$ Group showed no significant difference from placebo group. Serum parathyroid hormone (PTH) was also suppressed more efficiently by AAACa than $CaCO_3$.

Despite all these indirect lines of evidence indicating a high bioavailability of AAACa, a direct absorption test by a dual isotope method has not been conducted to date. We have therefore attempted to measure the fractional absorption of AAACa by using the dual stable-isotope method [4,5] to

compare it with $CaCO_3$ in subjects in the age group most likely to need effective calcium supplementation to maintain their bone health: postmenopausal women.

2. Experimental Section

2.1. Subjects

Ten postmenopausal women between 59 and 77 years of age (mean ± SD, 67 ± 5.3 years) leading a normal healthy daily life without any known disease possibly affecting bone and mineral metabolism volunteered to participate as test subjects in the present study by providing written consent (Table 1). One subject, shown in parenthesis in Tables 1 and 2, was dropped from analysis because of a measured fractional absorption (FA) value of 0% on giving $CaCO_3$. The Institutional Review Board of the Fujii Medical Clinic approved the study.

Table 1. Background of the test subjects.

No.	Age	Years after menopause	Height (cm)	Weight (kg)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
1	68	19	154	54	138	80
2	72	23	147	50	142	62
3	65	15	157	63	148	70
4	65	13	148	43	125	70
(5) *	(59)	(9)	(153)	(60)	(133)	(88)
6	59	8	152	58	152	85
7	65	13	151	56	150	85
8	77	28	150	48	142	80
9	64	15	145	50	140	90
10	71	19	148	48	122	70
Mean	67	17	150	52	139	76
SD	5.3	6.0	3.7	6.1	10.4	9.3

* Case No. 5 was not included in the statistical analysis.

2.2. Background Data of the Test Subjects

In order to assess the metabolic background of the test subjects, serum Ca, P, albumin, creatinine, BUN, 25(OH)vitamin D, intact parathyroid hormone (PTH), bone specific alkaline phosphatase (BAP), urinary N-terminal type I collagen fragments (NTx) and urinary calcium/ creatinine ratio (UCa/ Cr) were measured prior to the test. The laboratory tests related to bone and calcium metabolism gave results approximately within the normal range, as shown in Table 2, except for one subject, who had a serum 25(OH) vitamin D level in the insufficiency range (7.6 ng/mL). This subject was without symptoms and signs of vitamin D insufficiency such as hypocalcemia, hypophosphatemia, high alkaline phosphatase, muscle weakness and bone pain.

Table 2. Parameters of mineral and bone metabolism of the test subjects.

No.	Serum Ca	Serum P	Serum albumin	Serum creatinine	BUN	25(OH) vitamin D	Intact PTH	BAP	Urine NTx/Cr	Urine Ca/Cr
	mg/dL	mg/dL	g/dL	mg/dL	mg/dL	ng/dL	pg/dL	U/L	nMBCE/ mMCr	mg/mg
1	9.7	3.9	4.4	0.83	11.0	16.8	48	15.2	32.2	0.03
2	9.5	4.5	4.0	0.80	21.9	16.9	31	15.1	16.0	0.06
3	9.7	3.1	4.6	0.71	12.1	11.6	40	35.8	31.1	0.45
4	10.3	3.5	5.1	0.49	12.5	11.7	44	32.4	35.9	0.36
5 *	(9.5)	(3.4)	(4.5)	(0.74)	(14.2)	(21.8)	(61)	(21.6)	(29.0)	(0.08)
6	9.8	3.5	4.7	0.74	17.8	15.4	44	19.1	23.0	0.12
7	9.3	4.4	4.4	0.60	17.8	24.7	42	17.0	42.2	0.22
8	9.9	2.9	4.6	0.59	13.5	7.6	50	34.9	34.5	0.17
9	9.8	3.7	4.4	0.74	13.7	12.7	34	27.5	16.3	0.20
10	9.5	3.3	4.2	0.54	13.6	10.8	34	45.9	21.9	0.30
Mean	9.7	3.6	4.5	0.67	14.9	14.2	41	27.0	28.1	0.21
SD	0.29	0.55	0.31	0.119	3.53	4.95	6.59	11.02	9.20	0.138

Ca: calcium; P: phosphorus; BUN: Blood urea nitrogen; PTH: parathyroid hormone; BAP: Bone specific alkaline phosphatase; BCE: Bone collagen equivalent.

* Case No. 5 was not included in the statistical analysis

2.3. Materials

The first part of the test was performed on March 9, 2009, using ^{44}Ca-enriched $CaCO_3$ for oral load and ^{42}Ca in the form of $CaCl_2$, for intravenous injection (Table 3). On April 13, 2009, after one month, exactly the same procedure was repeated on the same test subjects, except for the use of ^{44}Ca-enriched AAACa in the place of $CaCO_3$ to ensure the stable isotope constituent of the body reached equilibrium. Intrinsic labeling is no doubt ideal, but it is impossible to label the shell of oysters abiding in the ocean, so an extrinsic labeling was adopted as the best substitute for it. The material for AAACa was obtained by heating oyster shell to 1,000 ^{o}C, resulting mostly in CaO powder after losing much of the organic components. To 5,082 mg of this CaO powder, 450.4 mg CaO Ca fraction was added that consisted of 95.9 ± 0.3% ^{44}Ca supplied by TRACE SCIENCES INTERNATIONAL (Ontario, Canada), and was thoroughly mixed in a melting pot. Aqueous solution of a small amount of algal component was pre-heated at a high temperature in a manner similar to the oyster shell to start a chemical reaction lasting for about 10 minutes. After sufficient stirring, it was divided into small portions for actual use and preserved in vacuum. The final product mostly consisted of $Ca(OH)_2$.

$CaCO_3$ labeled with ^{44}Ca was also obtained from the same source (the Ca fraction consisting of 95.9 ± 0.3% ^{44}Ca). To 781.6 mg of this material, 9,075 mg $CaCO_3$ (Japanese Pharmacopeia) was added, thoroughly mixed, and divided into small proportions and stored.

AAACa particle mean size was 5.8 microns; maximum size was 75 microns and $CaCO_3$ particle size ranged from 10 to 20 microns. As these values are based on different occasions of measurements they may not be directly comparable, but appears to lie over a similar range. If anything, a larger size is compatible with slower absorption.

For two subjects, part of the first urine sample was lost; in these cases, both parts of the test were repeated on July 30 and August 27, and the data from the uneventfully performed second set of tests were used to replace those of the first set.

The safety of the intravenous injection of $CaCl_2$ was verified before the study by the absence of any signs of toxicity such as chills, fever, neuromuscular irritability, skin eruptions, disturbance of consciousness, *etc.*

Table 3. Amount of isotope Ca (mg) per subject in 1 study.

Isotope	Oral						IV
	CaX			CaY			Total
	Supplied	Added	Total	Supplied	Added	Total	
^{42}Ca	0.01	1.79	1.80	0.00	1.79	1.79	3.192
^{43}Ca	0.01	0.39	0.40	0.00	0.39	0.39	0.0037
^{44}Ca	25.38	5.52	30.90	25.38	5.53	30.91	0.0334

The contents of Ca isotopes in the material used for the preparation of CaX and CaY on arrival from the supplier (Supplied), their contents in the material added to prepare samples for administration (Added) and the final total (Total) are indicated in Table 3.

A total of approximately 300 mg of Ca containing approximately 30 mg ^{44}Ca isotope (25 + 5) was orally administered to each subject and about 3 mg ^{42}Ca isotope was injected before the study and no symptoms and signs of toxicity were reported.

2.4. Test Procedure

After taking a fixed menu breakfast consisting of fruit juice, toast, eggs and coffee, a pre-test urine sample was collected (Urine 0) and ^{44}Ca-enriched $CaCO_3$ was orally administered followed by the intravenous injection of ^{42}Ca-enriched $CaCl_2$ 15 minutes later. A complete collection of 24 h urine followed (Urine 24). After one month to ensure clearance of the enriched isotope, exactly the same procedure, except for the use of ^{44}Ca-enriched AAACa instead of ^{44}Ca-enriched $CaCO_3$, was repeated.

2.4.1. Measurement of the Stable Isotope

Sample preparation for isotope enrichment measurement was conducted according to the method of Patterson *et al*. [6]. By using the inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 cs, Agilent Technologies, Inc., Tokyo), ^{42}Ca, ^{43}Ca, ^{43}Ca and other measurable stable Ca isotopes were measured in both Urine 0 and Urine 24. Utilizing ^{43}Ca as an internal standard of the stable Ca

isotopes, the ratio of each stable Ca isotope to ^{43}Ca was calculated. The increase of the $^{42}Ca/^{43}Ca$ and $^{44}Ca/^{43}Ca$ in Urine 24 above the pretest natural abundance level for each test subject over the corresponding value in Urine 0 was then obtained. By dividing the ratio of the actual amount of the enrichment of ^{44}Ca by the corresponding amount of the enrichment of ^{42}Ca from Urine 0 to Urine 24, the FA of the ^{44}Ca-enriched material was obtained; for $CaCO_3$ in the first part of the test and AAACa in the second part (Table 3).

2.5. Statistical Analysis

The Excel Statistical Package was used to compare the FA of $CaCO_3$ and AAACa by paired t-test. A correlation matrix among the FA data, age and parameters of bone and mineral metabolism was constructed and evaluated by the Spearman method in view of the inclusion of variables with uncertain distribution. The p values < 0.05 were considered significant.

3. Results and Discussion

As shown in Table 4, the mean Fractional absorption (FA) of AAACa, 23.1 ± 6.4%, was 1.57-times higher than the corresponding value of $CaCO_3$, 14.7 ± 6.4%, with a significant difference at p = 0.0060 determined using paired t-test.

Table 4. Fractional absorption (FA) of CaCO3 and AAACa by dual stable isotope method.

Subject	FA CaCO3	FA AAACa
1	7.5	21.1
2	20.0	29.7
3	21.9	34.7
4	19.6	20.4
(5) *	(0.0)	(18.8)
6	6.1	22.7
7	14.3	24.9
8	11.7	11.9
9	8.7	22.1
10	22.2	20.4
Mean	14.7	23.1
SD	6.4	6.4

Paired comparison between FA CaCO3 and FA AAACa

p = 0.0060, t = 3.708 (paired t-Test)

* Subject 5 was not included in the statistical analysis.

According to the evaluation by means of the correlation coefficient matrix (Spearman) (Table 5) among the parameters of bone and mineral metabolism summarized in Table 2, no significant correlation was found between the FA of either calcium carbonate or AAACa and each parameter. In the subject with the lowest serum 25(OH) vitamin D of 7.6 ng/mL, the FA of $CaCO_3$ value was medium in the group, *i.e.*, 11.7%, fifth from the lowest, and the FA of AAACa, 11.9%, was the lowest in the group.

Until the advent of the dual isotope method, the true FA of calcium was extremely difficult to measure due to the complex behavior of calcium in living organisms, such as the rapid exchange through multiple Ca pools and various pathways of exit and reentrance [7,8]. Utilizing the presence of multiple stable isotopes in nature, the dual stable isotope method was developed to circumvent this complexity, and it is the only method of directly measuring the fractional intestinal Ca absorption.

Abrams and coworkers as well as other investigators [12-23] have used this method extensively to estimate calcium absorption, establishing it as the gold standard for calcium absorption. Since calcium absorption is influenced by age and the state of bone, as well as mineral metabolism, a correlation matrix was constructed and evaluated by Spearman's method (Table 5). None of the metabolic parameters tested exhibited significant correlation with FA of the calcium compounds. Absence of significant correlation between FA of calcium compound and age was expected because of the narrow age range of this group.

The FA of Ca compounds obtained in this study of postmenopausal women, with a mean age of 66 years and with a tendency of low 25(OH) vitamin D, appears to be much lower than those observed in children and younger subjects: FA; 54.8–63.1% [21], 58.2–64.3% [22], and also younger postmenopausal women with mean age of 56: FA; 34.6–39.1% [23]. In healthy volunteers between 25 to 45 years much lower values, yet still higher than the results in the present study, were reported: FA; 26–31% [24]. The reduced FA in the current study subjects could also be due to reduced estrogen level after menopause. FA is, thus, markedly influenced by age. The age range of the test subjects was quite narrow in this group of subjects, unsuitable for the assessment of the age-FA correlation. Statistically, the tendency of age-FA correlation was non-significant.

Table 5. Spearman's correlation matrix and correlation coefficients among fractional absorption (FA) and parameters of bone and mineral metabolism.

	FA CaCO3	FA AAACa	Age	SCa	SP	Salb	Cre	BUN	25D	PTH	BAP	UNTx	UCa/Cr
FA CaCO3	1.0000	0.1423	0.4238	−0.3882	−0.1925	−0.3405	−0.4854	−0.0335	−0.3167	−0.5630	−0.4833	−0.1333	0.6333
FA AAACa		1.0000	−0.3490	−0.5297	0.4328	−0.2137	0.5042	0.3445	0.5690	−0.5232	−0.3766	−0.2762	0.0418

SCa: serum calcium; SP: serum phosphate; Salb: serum albumin; Cre: creatinine; BUN: blood urea nitrogen; 25D: 25(OH)vitaminD; PTH: parathyroid hormone; BAP: bone specific alkaline phosphatase; UNtx: urine N-terminal type I collagen fragments; UCa/Cr: urinary Ca/creatinine ratio

Although these subjects are reasonably homogeneous and apparently free of any comorbidity, which could potentially influence the test results, the present study is limited by the small number of test subjects. Unlike similar studies conducted in this field in the past, post-menopausal women – who need calcium supplementation most because of high risk of osteoporosis – were asked to participate. A rather low intra-group variation was encouraging, and a clear-cut difference in FA between the two test materials may also add to the credibility of the conclusion.

It is possible that the difference in molecular weight and physicochemical properties of the ^{44}Ca-enriched $CaCO_3$ and AAACa, mostly consisting of $Ca(OH)_2$ as the result of oxidation of $CaCO_3$

obtained from oyster shell, cannot be completely ruled out. The similar molecular size and comparable particle size actually measured as 5.8 to 75 for AAACa and 10 to 20 for $CaCO_3$ and this is a limitation of the result but should not have affected the primary outcome. In view of the similar molecular size and physicochemical properties between $CaCO_3$ and AAACa, both much smaller than organic Ca salts, however, confounding effect exerted on the calculation of the absorptive rate is rather unlikely and the conclusion of difference in the absorption rate between the two compounds should be reasonably supported.

4. Conclusions

This study aimed to compare the bioavailability of active absorbable algal calcium (AAACa), oyster shell powder heated to a high temperature, with an additional heated seaweed component (Heated Algal Ingredient, HAI), with that of calcium carbonate. The Fractional absorption of AAACa, (mean ± S.D.; 23.1 ± 6.4) was distinctly and significantly higher than that of $CaCO_3$ (14.7 ± 6.4; $p = 0.0060$ by paired t-test). The mean was approximately 1.57-times higher for AAACa than $CaCO_3$. Higher fractional absorption of AAACa compared with $CaCO_3$ supports previous reports on the more beneficial effect of AAACa than $CaCO_3$ on osteoporosis.

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References

1. Fujita, T.; Fukase, M.; Nakada, M.; Koishi, M. Intestinal absorption of oyster shell electrolysate. *Bone. Miner.* **1988**, *4*, 321-327.
2. Fujita, T.; Ohue, T.; Fujii, Y.; Miyauchi, Y.; Takagi, Y. Heated oyster shell-seaweed calcium (AAA-Ca) on osteoporosis. *Calcif. Tissue Int.* **1996**, *58*, 226-230.
3. Fujita, T.; Ohue, M.; Fujii, Y.; Miyauchi, A.; Takagi, Y. Reappraisal of Katsuragi Calcium Study, a prospective, double-blind, placebo-controlled study on vertebral deformity and fracture. *J. Bone Miner. Metab.* **2004**, *22*, 32-38.
4. Abrams, S.A.; Esteban, N.V.; Vieila, N.V.; Yergey, A.L. Dual tracer stable isotopic assessment of calcium absorption and endogenous fecal excretion in low birth weight infants. *Pediatr. Res.* **1991**, *29*, 615-618.
5. Abrams, S.A. Using stable isotopes to assess mineral absorption and utilization by children. *Am. J. Clin. Nutr.* **1999**, *70*, 955-964.
6. Patterson, K.Y.; Veillon, C.; Hill, A.D.; Moser-Veillon, P.B.; O'Haver, T.C. Measurement of calcium stable isotope tracers using cool plasma ICP-MS. *J. Anal. At. Spectr.* **1999**, *14*, 1673-1677.

7. Yergey, A.L.; Vieira, N.E.; Couell, D.G. Direct measurement of dietary fractional absorption using calcium isotopic tracers. *Biomed. Environ. Mass. Spectr*. **1987**, *14*, 603-607.
8. Hillman, L.S.; Tack, E.; Covell, D.G.; Vieina, N.E.; Yergey, A.L. Measurement of true calcium absorption in premature infants using intravenous 46Ca and oral 44Ca. *Pediatr. Res*. **1988**, *23*, 589-594.
9. Abrams, S.A. Assessing mineral metabolism in children using stable isotopes. *Pediatr. Blood Cancer* **2008**, *50*, 438-441.
10. Abrams, S.A.: Griffin, I.J.; Hawthorne, K.M. Chen, Z.; Gunn, S.K. Wilde, M.; Darlington, G.; Shypailo, R.J.; Ellis, K.J. Vitamin D receptor Fok1 polymorphisms affect calcium absorption, kinetics, and bone mineralization rates during puberty. *J. Bone. Miner. Res*. **2005**, *20*, 945-953.
11. Abrams, S.A. Using stable isotopes to assess the bioavaila bility of minerals in food-fortification programs. *Forum Nutr*. **2003**, *56*, 312-313.
12. Vargas Zapata, C.L.; Donangelo, C.M.; Woodhouse, L.R.; Abrams, S.A.; Spencer, E.M. King, J.C. Calcium homeostasis during pregnancy and lactation in Brazilian women with low calcium intakes: a longitudinal study. *Am. J. Clin. Nutr*. **2004**, *80*, 417-422.
13. Abrams, S.A.; Griffine, I.J. Hicks, P.D. Gunn, S.K. Pubertal girls only partially adapt to low dietary calcium intakes. *J. Bone Miner. Res*. **2004**, *19*, 757-763.
14. Abrams, S.A. Griffin, I.J.; Herman, S. Using stable isotopes to assess the bioauailabilty of mineral in food fortification programs. *Food Nutr. Bull.* **2002**, *23*, 158-165.
15. Abrams, S.A. Calcium turnover and nutrition through the life cycle. *Proc. Nutr. Soc*. **2001**, *60*, 283-289.
16. Abrams, S.A. Using stable isotope to assess mineral absorption and utilization by children. *Am. J. Clin. Nutr*. **1999**, *70*, 955-964.
17. Miller, J.Z.; Smith, D.L.; Flora, L.; Peacock, M.; Johnston, C.C., Jr. Calcium absorption in children estimated from single and double stable calcium isotope techniques. *Clin. Chim. Acta*. **1989**, *183*, 107-113.
18. Ames, S.K.; Ellis, K.J.; Gunn, S.K.; Copeland, K.C.; Abrams, S.A. Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. *Bone Miner. Res*. **1999**, *14*, 740-746.
19. Eastell, R.; Vieira, N.E.; Yergey, A.L.; Riggs, B.L. One day test using stable isotopes to measure true fractional calcium absorption. *J. Bone Miner. Res*. **1989**, *4*, 463-468.
20. Fairweather – Tait, S.; Johnson, A.; Eagles, J.; Ganatra, S.; Kennedy, H.; Gurr, MI. Studies on calcium absorption from milk using a double label stable isotope technic. *Br. J. Nutr*. **1998**, *62*, 379-388.
21. Lee, W.T.; Leung, S.S.; Fairweather-Tait, S.J.; Leung, D.M.; Tsang, H.S.; Eagles, J.; Fox, T.; Wang, S.H.; Xu, Y.C.; Zeng, W.P. Ture fractional calcium absorption in Chinese children measured with stable isotope(42Ca and 44Ca). *Br. J. Nutr*. **1994**, *72*, 883-897.
22. Lee, W.; Leung, S.S.; Xu, Y.C.; Wang, S.H.; Zeng, W.P.; Lau, J.; Fairweather-Tait, S.J. Effects of double-blind controlled calcium supplementation on calcium absorption in Chinese children measured with stable isotopes (42Ca and 44Ca). *Br. J. Nutr*. **1995**, *73*, 311-321.

23. Tanimoto, H.; Fox, T.; Eagles, J.; Satoh, H.; Nazawa, H.; Okiyama.; Morinaga, Y.; Fairweather–Tait, S. Acute effects of poly-gamma-glutamic avid on calcium absorption in postmenopausal women. *J. Am. Coll. Nutr*. **2007**, *26*, 645-649.
24. van der Hee, R.M.; Miret, S.; Slettenaar, M.; Duchateau, G.S.; Rietveld, A.G.; Wikinson, J.E.; Quail, P.J.; Berry, M.J.; Dainty, J.R.; Teucher, B.; Fairweather-Tait, S.J. Calcium absorption from fortified ice cream formulations compared with calcium absorption from milk. *J. Am. Diet Assoc.* **2009**, *109*, 830-835.

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ORIGINAL ARTICLE

High fruit intake is associated with a lower risk of future hypertension determined by home blood pressure measurement: the OHASAMA study

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We investigate associations of fruit and vegetable intake with the risk of future hypertension using home blood pressure in a general population from Ohasama, Japan. We obtained data from 745 residents aged ⩾35 years without home hypertension at baseline. Dietary intake was measured using a validated 141-item food frequency questionnaire, and subjects were then divided into quartiles according to the fruit and vegetable intake. Home hypertension was defined as home systolic/diastolic blood pressure of ⩾135/85 mm Hg and/or the use of antihypertensive medication. During a 4-year follow-up period, we identified 222 incident cases of home hypertension. After adjustment for all putative confounding factors, the highest quartile of fruit intake was associated with a significantly lower risk of future home hypertension (odds ratio 0.40, 95% confidence interval 0.22–0.74, $P=0.004$). In conclusion, this study, based on home blood pressure measurement, suggests that higher intake of fruit is associated with a lower risk of future home hypertension.
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Keywords: fruit intake; vegetable intake; nutrition; home blood pressure; home hypertension; healthy community resident

Introduction

Hypertension is a major cause of morbidity and mortality,[1] with many studies indicating it to be significantly associated with an increased risk of cardiovascular disease (CVD) events.[2,3]

For several decades, researchers have mainly focused on the potentially adverse or preventive effects of various dietary factors.[4–16] Among these, fruit and vegetable intake has a especially powerful association with lower blood pressure (BP), and was found to reduce the risk of hypertension.[4–7] Our previous study using self-measured BP at home (home BP) found a significant cross-sectional association between intake of fruit and risk of hypertension.[17] However, although most studies exclude subjects who report a dietary change resulting from a diagnosis, the cross-sectional studies cannot remove subjects who change their diet after the diagnosis of hypertension.

Furthermore, in the majority of these studies, the definition of hypertension was based on conventional BP measurements. Because of the white-coat effect, a condition characterized by an elevated BP reading in a medical setting, these studies often overestimate the risk of high BP.[18] On the other hand, home BP measurements enable researchers to obtain multiple measurements over a long observation period under relatively controlled conditions.[19–22] The main strength of home BP is that it is not influenced by observer and regression dilution biases or the white-coat effect. Because of these benefits, home BP measurements are now

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considered a more accurate and reliable way of reflecting target organ damage and the prognosis of CVD when compared with conventional BP measurement taken in a medical setting,[19–22] and are also recommended in several general hypertension guidelines.[23,24]

Moreover, as there are geographical differences in the types of food intake and risk factors among countries,[25,26] it is important to confirm the reproducibility of previous findings of associations between BP and food and nutrient components in each population.

The aim of this study was to examine the association of fruit and vegetable intake with the risk of hypertension diagnosed by home BP during 4 years of follow-up in a Japanese general population.

Subjects and methods

Design

This study was part of the Ohasama study, a longitudinal community-based observational study of individuals who have participated in a home BP measurement project in Ohasama, Iwate prefecture, Japan. The geographic and demographic characteristics of the study subjects have been reported previously.[19,27]

This study was approved by the institutional review board of Tohoku University School of Medicine and by the Department of Health of the Ohasama Town Government. Subjects provided written informed consent to participate.

Study population

In 1998, there were 5081 individuals aged $\geqslant 35$ years in Ohasama. Of the 4628 who answered the questionnaire (response rate 91.1%), 1820 subjects took part in home BP measurement; these individuals collected their own BP data on at least 3 days during the 4-week measurement period in 1998. Among those, people who had home hypertension at baseline ($n=394$) and those who died ($n=43$) or moved away from the town ($n=5$) before the follow-up measurements were excluded from the study. Of the remaining 1378 eligible individuals, 805 subjects (58%) took part in the follow-up home BP measurements.

In addition, 60 subjects were excluded for the following reasons: those who took <3 home BP measurements at follow-up ($n=20$) and those who had extreme levels of energy intake (in the upper or lower 2.5% of the range for all subjects: $n=40$). Finally, data from 745 subjects who were normotensive on baseline home BP (274 men and 471 women) were analysed. Compared with those who were ultimately excluded based on the exclusion criteria of the 1378 eligible individuals, the 745 participants who completely fulfilled the study criteria were more likely to be men, and of older age.

Home BP measurement

Baseline home BP was measured using the HEM701C monitor (Omron Healthcare Co., Ltd, Kyoto, Japan), a semiautomatic device based on the cuff-oscillometric method, which generates a digital display of both systolic BP and diastolic BP. We used HEM747ICN devices (Omron) for follow-up measurements. Both devices have been validated[28] and satisfy the criteria of the Association for the Advancement of Medical Instrumentation (the HEM747ICN is exactly same as the Omron HEM735C except that the latter does not incorporate an integrated circuit memory). As the circumference of the arm was <34 cm in most cases, we used a standard arm cuff in all cases.[27] In this study, home BP was defined as the mean of all first measurements recorded during the 4-week period. The mean ($\pm$ s.d.) number of home BP measurements was 23 ± 6. Hypertension was defined as use of antihypertensive medication and/or home BP values of $\geqslant 135/85$ mm Hg at follow-up measurement.[20–24]

Food frequency questionnaire

Standardized methodology was used to calculate fruit and vegetable intake from data obtained in a Japanese version of a food frequency questionnaire. The reproducibility and validity of this questionnaire were previously reported in detail.[29,30] The questionnaire asked about the average frequency of intake of each food during the previous year according to nine frequency categories ranging from no consumption to $\geqslant 7$ times per day. A standard portion size of one serving was specified for each food, and respondents were asked whether their usual portion was larger (>1.5 times), the same or smaller (<0.5) than the standard. In this study, we took into account energy from food sources of alcohol; for example, seasonings that include alcohol. However, we did not consider alcohol derived from alcoholic drinks such as beer and wine in the total energy count because we treated such alcohol intake as a separate variable. Nutritional supplements were not taken into account because there were few supplement users.

All food and nutrient intakes were adjusted for total energy intake using the residual method,[31–33] and separate regression models were performed to obtain the residuals for men and women. Following this procedure, subjects were divided into quartiles according to the intake of fruit and vegetables. In this study, the lowest quartiles were used as reference categories.

Statistical analysis

To examine how the intake of fruit or vegetables was associated with the risk of future home hypertension defined on the basis of home BP measurement, we used multiple logistic regression analyses after adjustment for other putative confounding factors

related to hypertension. These were gender (men/women), age (continuous), body mass index (BMI; $<25/\geqslant 25\,kg\,m^{-2}$), frequency of exercise (rarely or never, 1 or 2 h per week and >3 h per week), smoking status (never, past or current smoker), alcohol consumption (rarely or never, <540 ml of sake per day and ⩾540 ml of sake per day: 540 ml of sake = 81 g of alcohol), energy-adjusted fat intake and sodium intake (continuous), baseline systolic home BP (continuous) and a history of diabetes, hypercholesterolaemia and CVD (yes/no).

Moreover, we stratified the analysis by lifestyle factors, such as overweight ($BMI = 25\,kg/m^{-2}$ as cutoff), frequency of exercise, smoking status and alcohol consumption, to explore associations related to these factors. We tested interactions by introducing a multiplicative term into the main effect models. We also examined the combined effects of risk factors and fruit or vegetable intakes. For all analyses, statistical significance was defined as a two-tailed *P*-value of <0.05. All analyses were conducted using SPSS software version 14 for Windows (SPSS Inc., Chicago, IL, USA).

Results

At the time of the follow-up measurements, 222 subjects (29.8%) had developed home hypertension (mean duration of follow-up: 4.1 years). Among these, 70 were defined as having home hypertension because they had started treatment with antihypertensive medication.

The distributions of characteristics across quartile of each fruit and vegetable intake at baseline are shown in Table 1. In the study, the most commonly consumed type of fruit was citrus fruit ($18.5\,g\,day^{-1}$), followed by apple ($8.6\,g\,day^{-1}$), grape ($5.9\,g\,day^{-1}$) and watermelon ($5.6\,g\,day^{-1}$). Compared with those in the highest quartile of fruit intake, those in the lowest quartile were more likely to be men, of younger age, current smokers, heavier drinkers and with lower diastolic home BP. Subjects with the highest quartile of fruit intake tended to consume less energy and carbohydrate and more sodium, and fruit- and vegetable-related nutrients (that is, potassium, magnesium, β-carotene, folate, vitamin C and total dietary fibre) than subjects in the lowest quartile. As for food intake, the highest intake of fruit was associated with low intakes of rice, bread and noodles, and with high intakes of vegetables and seaweeds. Compared with those with highest quartile of fruit intake, subjects with quartile 3 of fruit intake reported more fat and less protein and calcium intake (table not shown, intake in quartile 3; fat, 42.7 ± 0.8 g; protein, 61.6 ± 0.6 g; and calcium 630 ± 14 mg). We observed similar tendencies for vegetable intake. In each category, the frequency of exercise and incidence of home hypertension at follow-up did not differ.

Table 2 shows the association between fruit and vegetable intake and the risk of future home hypertension. In the sex- and BMI-adjusted analysis, the highest quartile of fruit intake was associated with a significantly lower risk for future home hypertension (odds ratio compared with the lowest quartile for fruit intake: 0.44; $P=0.005$), whereas no association was observed for vegetable intake. After adjustment for putative confounding factors, these associations did not change. Compared with the lowest quartile for intake of fruit, a 60.0% lower risk of hypertension was found in those with the highest quartile of fruit intake ($P=0.004$). Further adjustment for putative confounding factors, vegetables and related nutrients (potassium, β-carotene, folate, vitamin C and total fibre) attenuated these results, but there was still a significantly lower risk of future home hypertension in the highest quartile of fruit intake (odds ratio = 0.45; $P=0.025$).

Regarding joint classification of quartiles of fruit intake and risk factors for home hypertension, Figure 1 shows the risk associated with BMI status at each quartile of fruit intake. The odds ratio for the comparison of overweight with highest quartile of fruit intake to overweight with lowest quartile of fruit intake was 0.21 ($P=0.005$). There was no significant interaction between BMI and fruit intake ($P>0.10$). When we adjusted for baseline diastolic home BP instead of systolic home BP, the results were almost the same.

Discussion

This study indicated that high fruit intake is strongly associated with a lower risk of future home hypertension. The inverse association between fruit intake and future home hypertension was persistent among subgroups of overweight and normal-weight individuals.

Our study has several strengths. It is the first to examine whether fruit and vegetable intake predicts hypertension measured by home BP. Measuring BP at home can eliminate several biases, such as the white-coat effect,[20–22] and therefore the results might more accurately determine the relationship between BP and fruit and vegetable intake. Because of the prospective design and exclusion of hypertensive subjects at baseline, we believe we could minimize the number of subjects who changed their diet because of a diagnosis of high BP.

The second strength is that, to the best of our knowledge, this is the first study to clarify the association between fruit and vegetable intake and future hypertension in Asian subjects.

Fruit/vegetable intake and future home hypertension
We found that high fruit intake was linked to a lower risk of future home hypertension, whereas no association was observed for high vegetable intake.

Table 1 Distribution of characteristics across quartiles of fruit and vegetable intake ($n = 745$)

	Quartile of fruit consumption		P-*value*	*Quartile of vegetable consumption*		P-*value*
	1 (n = *187*)	*4* (n = *186*)		*1* (n = *187*)	*4* (n = *186*)	
Baseline						
Gender (men %)	51.3	26.9	<0.0001	62.6	18.3	<0.0001
Age	55.3 ± 0.8	57.4 ± 0.8	0.057	54.2 ± 0.8	59.0 ± 0.8[a]	<0.0001
Alcohol consumption (%)			0.010			<0.0001
Rarely or never	65.8	82.3		68.4	83.3	
<540 ml of sake per day	28.3	16.1		27.3	15.1	
⩾540 ml of sake per day	5.9	1.6		4.3	1.6	
Current smokers (%)	27.8	13.4	0.001	27.8	9.1	<0.0001
Exercise (rarely or never %)	76.5	83.3	0.290	79.7	80.1	0.643
Body mass index (kg m^{-2}, ⩾25%)	21.9	24.2	0.925	20.9	28.0	0.214
Home BP (mm Hg)						
Systolic	115.8 ± 0.7	113.6 ± 0.8	0.273	115.8 ± 0.7	114.3 ± 0.8	0.403
Diastolic	73.2 ± 0.5	71.3 ± 0.5	0.014	73.6 ± 0.5	71.2 ± 0.5[a]	0.002
Mean intakes of food and nutrients[b]						
Rice, bread and noodles (g)	566 ± 5	479 ± 5[a]	<0.0001	685 ± 15	397 ± 10[a]	<0.0001
Sugar (g)	6.6 ± 0.4	8.9 ± 0.4	<0.0001	4.3 ± 0.3	11.7 ± 0.6[a]	<0.0001
Nuts (g)	101.7 ± 4.7	73.6 ± 4.7	0.057	80.7 ± 7.5	100.2 ± 4.5[a]	0.021
Pulses (g)	2.7 ± 0.5	5.0 ± 0.5[a]	0.008	2.3 ± 0.3	4.7 ± 0.6[a]	0.001
Vegetables (g)	198.8 ± 8.4	249.2 ± 8.3[a]	<0.0001	90.3 ± 3.7	390.9 ± 8.6[a]	<0.0001
Seaweeds (g)	18.2 ± 1.1	22.8 ± 1.1[a]	0.009	13.4 ± 1.2	29.2 ± 1.4[a]	<0.0001
Fish and shellfish (g)	65.1 ± 3.1	58.5 ± 3.1	0.143	47.2 ± 4	68.6 ± 2.6[a]	<0.0001
Meats (g)	20.5 ± 1	17.2 ± 1.0	0.052	13.9 ± 0.9	22.7 ± 1.5[a]	<0.0001
Eggs (g)	29.5 ± 1.2	27.8 ± 1.2	0.744	22.6 ± 1.2	31.1 ± 1.5[a]	<0.0001
Dairy products (g)	220 ± 13	238 ± 13	0.497	241 ± 17	212 ± 12	0.166
Energy (kcal)	2163 ± 42	1952 ± 41	<0.0001	2135 ± 38	1897 ± 51[a]	<0.0001
Protein (g)	64.1 ± 0.6	62.3 ± 0.6	0.029	56.7 ± 1	68.2 ± 0.9[a]	<0.0001
Fat (g)	38.8 ± 0.9	40.1 ± 0.8	0.009	34.0 ± 0.9	45.3 ± 0.9[a]	<0.0001
Carbohydrates (g)	299.4 ± 0.7	305.9 ± 0.7[a]	<0.0001	336.8 ± 3.8	278.7 ± 2.8[a]	<0.0001
Sodium (mg)	4264 ± 151	5181 ± 150[a]	<0.0001	2959 ± 131	6610 ± 191[a]	<0.0001
Potassium (mg)	2396 ± 40	2797 ± 40[a]	<0.0001	1879 ± 23	3346 ± 48[a]	<0.0001
Calcium (mg)	631 ± 14	661 ± 14	0.332	549 ± 20	747 ± 17[a]	<0.0001
Magnesium (mg)	287 ± 1.5	313.7 ± 4.5[a]	0.001	232.8 ± 5.2	370 ± 5.2[a]	<0.0001
β-carotene (μg)	2806 ± 138	3686 ± 137[a]	<0.0001	1477 ± 56	5211 ± 202[a]	<0.0001
Folate (μg)	299.5 ± 8.5	335.3 ± 8.5[a]	0.026	205.4 ± 6.7	443.3 ± 10.7[a]	<0.0001
Vitamin C (mg)	59.9 ± 2.3	97.7 ± 2.3[a]	<0.0001	44.2 ± 1.7	111.4 ± 3.0[a]	<0.0001
Total dietary fibre (g)	14.8 ± 0.4	17.7 ± 0.4[a]	<0.0001	10.3 ± 0.3	23.1 ± 0.5[a]	<0.0001
Follow-up[c]						
Antihypertensive medication (%)	9.1	7.0	0.250	4.3	11.3	0.037
Home BP (mm Hg)						
Systolic	126.4 ± 1.1	123.9 ± 1.1	0.212	124.8 ± 1.1	124.4 ± 1.1	0.835
Diastolic	75.5 ± 0.7	73.4 ± 0.6	0.056	74.9 ± 0.7	72.8 ± 0.6	0.127
Home hypertension (%)[d]	34.8	24.2	0.076	31.6	26.9	0.450

Continuous variables are presented as mean ± s.e.
One-way analysis of variance (ANOVA) was used for continuous variables and χ^2 test for categorical variables, comparing quartiles of each food group.
[a]Statistical significance was defined as $P<0.05$ compared with quartile 1 (lowest) using Bonferroni *post hoc* test.
[b]Data were adjusted for total energy by the residual method.
[c]Mean duration of the period between the baseline and the follow-up home blood pressure (home BP) was 4.1 ± 0.7.
[d]Home hypertension was defined as use of antihypertensive medication and/or home BP values of ⩾135/85 mm Hg at follow-up.

Results of some studies, which examined the association between combined fruit and vegetable intake and risk of hypertension, are partially consistent with the present findings,[4–6,34] but no studies have shown significant inverse associations between intake of fruit alone and the risk of hypertension. Other studies have reported a significant protective association between intake of fruit and risk of CVD.[15,16] Our results are consistent with this.

Other related nutrients and home hypertension

We also analysed the intakes of potassium, folate, magnesium, vitamin C and β-carotene, which are highly correlated with fruit and vegetable intake. However, these dietary factors were not associated with the risk of home hypertension. Moreover, adjustment for these dietary factors did not significantly modify the findings. In this study, the most commonly consumed type of fruit was citrus fruit, followed by apple, grape and watermelon. Although

Table 2 Adjusted odds ratio (95% confidence interval) for the association between fruit and vegetable intake and the risk of future home hypertension ($n = 745$)

	Quartile 1	*Quartile 2*	*Quartile 3*	*Quartile 4*	P *for trend*
Fruit (g day^{-1})[a]	⩽38.40	38.41–63.80	63.8–100.02	100.03⩽	
Adjusted[b]	1.00	0.74 (0.44–1.26)	0.85 (0.80–1.44)	0.44 (0.25–0.78)	0.033
Adjusted[c]	1.00	0.64 (0.36–1.15)	0.70 (0.39–1.26)	0.40 (0.21–0.74)	0.037
Vegetables (g day^{-1})[a]	⩽143.41	143.42–211.55	211.56–282.75	282.76⩽	
Adjusted[b]	1.00	0.90 (0.52–1.57)	1.28 (0.74–2.23)	0.71 (0.40–1.24)	0.168
Adjusted[c]	1.00	0.96 (0.52–1.75)	1.11 (0.60–2.05)	0.75 (0.40–1.38)	0.597

[a]Data were adjusted for total energy by the residual method.
[b]Adjusted for age, gender and body mass index (BMI).
[c]Adjusted for age, gender, BMI, frequency of exercise, smoking status, alcohol consumption, energy-adjusted fat and sodium consumption, baseline systolic home BP, and a past history of diabetes, hypercholesterolaemia and cardiovascular disease.

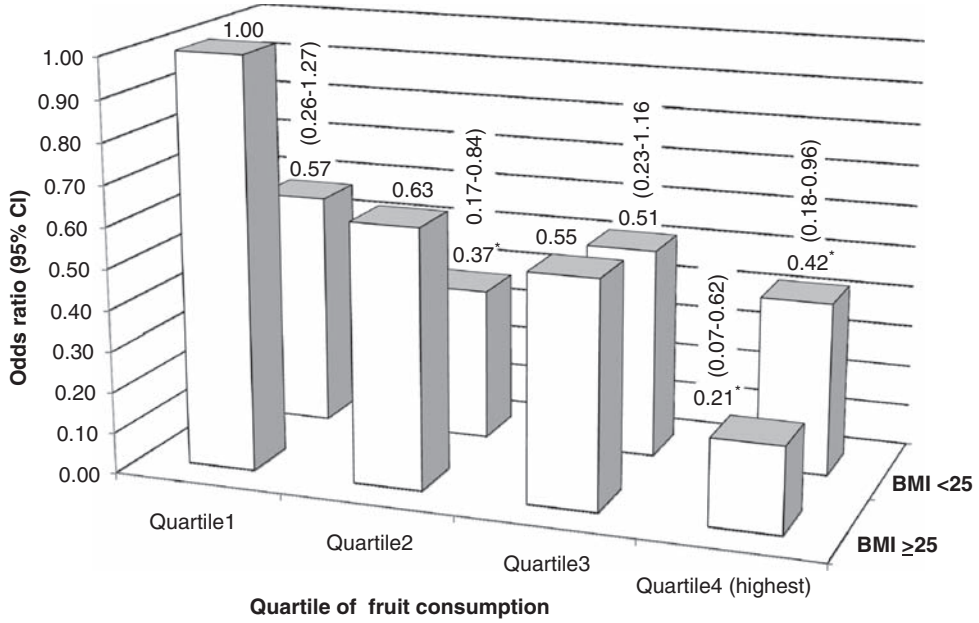

Figure 1 Multivariate odds ratio of home hypertension according to joint classifications of fruit intake and body mass index (BMI). The category of lowest fruit intake and the overweight (BMI of ⩾25) was used as reference. Model adjusted for age, gender, frequency of exercise, smoking status, alcohol consumption, energy-adjusted fat and sodium consumption, baseline systolic home BP and a past history of diabetes, hypercholesterolaemia and cardiovascular disease. *$P<0.05$.

individual dietary factors, such as vitamin C and folate, were not associated with risk of home hypertension, it is possible that the total balance of these factors in these commonly consumed fruits might be useful for the prevention of future hypertension.

Characteristics of fruit intake

These result, however, showed a lack of continuity in terms of risk of hypertension for each quartile of fruit intake, despite the significant association between the highest quartile of fruit intake and a lower risk of hypertension. Subjects in the third quartile of fruit intake reported higher fat and lower protein intake than those in the highest quartile, and thus the risk of hypertension might be influenced by these factors.

We also found no association between the highest quartile intake of vegetables and risk of hypertension. This study confirmed the findings of our previous cross-sectional study,[17] in that those who consumed more fruit and vegetables had higher sodium and fat intake. This might be attributable to seasonings, including soy sauce and table salt, and methods of cooking vegetables, such as deep frying. The higher fat and sodium intake among those who consumed more fruit might be attributable to the close correlation between the intake of fruit and vegetables. A number of factors, such as lifestyle, food availability, food culture and dietary habits, might also be related to BP and risk of hypertension.

In this study, we found significant differences in dietary characteristics across quartiles of fruit intake after adjusting for all putative confounding factors. Compared with subjects with higher intake of fruit,

those with the lowest intake of fruit consumed more carbohydrate-containing foods and meat, and less vegetables and seaweed. It therefore seems that a higher intake of fruit was associated with a healthier diet. As people consume diets consisting of a variety of foods with complex combinations of nutrients, the examination of only single foods could result in the identification of erroneous associations between dietary factors and disease. Furthermore, the risk of hypertension could be attributable to other food groups. When several nutrients with small BP-lowering effects are consumed together, the cumulative effects may be sufficient for detection. The dietary pattern approach using factor and cluster analyses[35] could provide more information regarding risk of home hypertension in further studies.

Study limitations

Several limitations of this study need to be discussed. First, information regarding food and nutrient intake in this study was obtained on the basis of dietary recall. The correlation between the food frequency questionnaire and usual diet has been well established, but there are several problems, for example, limited number of items and minimal information about portion size.

Second, we did not find a significant interaction between fruit consumption and BMI. However, a gradient declining risk of home hypertension with increasing fruit intake was apparent only for the overweight subjects, suggesting that such an interaction may have been present. Therefore, it is possible that the lack of statistical significance was because of the small size of the eight subgroups. Larger studies would be needed to clarify the presence of a statistically significant interaction between fruit consumption and BMI.

The possibility of selection bias also needs to be considered when generalizing the present findings, because only 54.1% of those eligible to participate in the study agreed to take part. As we excluded those who had home hypertension at baseline, this could mean that healthy people were more likely to be followed up. However, although the nonparticipants were older and had higher energy intake than those who participated in the study, other lifestyle factors did not differ significantly between participants and nonparticipants. Marked differences also exist in the epidemiology of home hypertension between Japan and Western countries;[36] thus, further research in other ethnic and cultural populations is needed to confirm the generalizability of our findings.

In this study, a higher intake of fruit was associated with a healthier lifestyle such as limited alcohol intake and avoidance of smoking. Therefore, although we adjusted for these confounding factors, it is possible that other factors associated with healthier lifestyle not measured in this study might confound the findings. Further studies with more detailed information on lifestyle-associated factors are required to further investigate the association observed in this study.

Conclusions

The present results from the Ohasama study suggest that high intake of fruit is potentially associated with a lower risk of future home hypertension. Although the mechanism for BP lowering through fruit and vegetable intake remains unclear,[37,38] selective intake of healthy foods and nutrients may prevent hypertension. Using home BP in general subjects enable to be considered highly health consciousness and subsequent early dietary intervention is expected to prevent hypertension and CVD.

What is known about this topic

- Fruit and vegetable intake has a powerful association with lower blood pressure and was found to reduce the risk of hypertension.
- But there are geographical differences in the types of food intake and risk factors among countries, and the relationship of diet with blood pressure in Asian populations has not been fully investigated.
- Furthermore, there is no study to examine the association between fruit and vegetable intake and the risk of future home hypertension determined by home blood pressure measurement.

What this study adds

- Higher intake of fruit is associated with a lower risk of future home hypertension.
- Higher intake of fruit is also associated with a healthier diet.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet* 2005; **365**: 217–223.
2 Lawes CM, Rodgers A, Bennett DA, Parag V, Suh I, Ueshima H *et al.* Blood pressure and cardiovascular disease in the Asia Pacific region. *J Hypertens* 2003; **21**: 707–716.
3 Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 2002; **360**: 1903–1913.
4 Ascherio A, Hennekens C, Willett WC, Sacks F, Rosner B, Manson J *et al.* Prospective study of nutritional factors, blood pressure, and hypertension among US women. *Hypertension* 1996; **27**: 1065–1072.
5 Ascherio A, Rimm EB, Giovannucci EL, Colditz GA, Rosner B, Willett WC *et al.* A prospective study of nutritional factors and hypertension among US men. *Circulation* 1992; **86**: 1475–1484.
6 Stamler J, Liu K, Ruth KJ, Pryer J, Greenland P. Eight-year blood pressure change in middle-aged men: relationship to multiple nutrients. *Hypertension* 2002; **39**: 1000–1006.
7 Miura K, Greenland P, Stamler J, Liu K, Daviglus ML, Nakagawa H. Relation of vegetable, fruit, and meat intake to 7-year blood pressure change in middle-aged men: the Chicago Western Electric Study. *Am J Epidemiol* 2004; **159**: 572–580.
8 Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM *et al.* A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med* 1997; **336**: 1117–1124.
9 Lairon D, Arnault N, Bertrais S, Planells R, Clero E, Hercberg S *et al.* Dietary fiber intake and risk factors for cardiovascular disease in French adults. *Am J Clin Nutr* 2005; **82**: 1185–1194.
10 Psaltopoulou T, Naska A, Orfanos P, Trichopoulos D, Mountokalakis T, Trichopoulou A. Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Am J Clin Nutr* 2004; **80**: 1012–1018.
11 Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D *et al.* Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med* 2001; **344**: 3–10.
12 Srinath Reddy K, Katan MB. Diet, nutrition and the prevention of hypertension and cardiovascular diseases. *Public Health Nutr* 2004; **7**: 167–186.
13 Svetkey LP, Simons-Morton D, Vollmer WM, Appel LJ, Conlin PR, Ryan DH *et al.* Effects of dietary patterns on blood pressure: subgroup analysis of the Dietary Approaches to Stop Hypertension (DASH) randomized clinical trial. *Arch Intern Med* 1999; **159**: 285–293.
14 Whelton PK, He J, Appel LJ, Cutler JA, Havas S, Kotchen TA *et al.* Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. *JAMA* 2002; **288**: 1882–1888.
15 Takachi R, Inoue M, Ishihara J, Kurahashi N, Iwasaki M, Sasazuki S *et al.* Fruit and vegetable intake and risk of total cancer and cardiovascular disease: Japan Public Health Center-Based Prospective Study. *Am J Epidemiol* 2008; **167**: 59–70.
16 Hung HC, Joshipura KJ, Jiang R, Hu FB, Hunter D, Smith-Warner SA *et al.* Fruit and vegetable intake and risk of major chronic disease. *J Natl Cancer Inst* 2004; **96**: 1577–1584.
17 Utsugi M, Ohkubo T, Kikuya M, Kurimoto A, Sato R, Suzuki K *et al.* Fruit and vegetable consumption and the risk of hypertension determined by self measurement of blood pressure at home: the Ohasama study. *Hypertens Res* 2008; **31**: 1435–1443.
18 Pickering TG, James GD, Boddie C, Harshfield GA, Blank S, Laragh JH. How common is white coat hypertension? *JAMA* 1988; **259**: 225–228.
19 Ohkubo T. Prognostic significance of variability in ambulatory and home blood pressure from the Ohasama study. *J Epidemiol* 2007; **17**: 109–113.
20 Parati G, Stergiou GS, Asmar R, Bilo G, de Leeuw P, Imai Y *et al.* European Society of Hypertension guidelines for blood pressure monitoring at home: a summary report of the Second International Consensus Conference on Home Blood Pressure Monitoring. *J Hypertens* 2008; **26**: 1505–1526.
21 Imai Y, Otsuka K, Kawano Y, Shimada K, Hayashi H, Tochikubo O *et al.* Japanese society of hypertension (JSH) guidelines for self-monitoring of blood pressure at home. *Hypertens Res* 2003; **26**: 771–782.
22 Pickering TG, Miller NH, Ogedegbe G, Krakoff LR, Artinian NT, Goff D. Call to action on use and reimbursement for home blood pressure monitoring: executive summary: a joint scientific statement from the American Heart Association, American Society of Hypertension, and Preventive Cardiovascular Nurses Association. *Hypertension* 2008; **52**: 1–9.
23 Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G *et al.* 2007 Guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2007; **28**: 1462–1536.
24 Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL *et al.* The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003; **289**: 2560–2572.
25 Dauchet L, Ferrieres J, Arveiler D, Yarnell JW, Gey F, Ducimetiere P *et al.* Frequency of fruit and vegetable consumption and coronary heart disease in France and Northern Ireland: the PRIME study. *Br J Nutr* 2004; **92**: 963–972.
26 Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part II: variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies. *Circulation* 2001; **104**: 2855–2864.
27 Imai Y, Satoh H, Nagai K, Sakuma M, Sakuma H, Minami N *et al.* Characteristics of a community-based distribution of home blood pressure in Ohasama in northern Japan. *J Hypertens* 1993; **11**: 1441–1449.

28 Imai Y, Abe K, Sasaki S, Minami N, Munakata M, Sakuma H *et al.* Clinical evaluation of semiautomatic and automatic devices for home blood pressure measurement: comparison between cuff-oscillometric and microphone methods. *J Hypertens* 1989; **7**: 983–990.
29 Ogawa K, Tsubono Y, Nishino Y, Watanabe Y, Ohkubo T, Watanabe T *et al.* Validation of a food-frequency questionnaire for cohort studies in rural Japan. *Public Health Nutr* 2003; **6**: 147–157.
30 Tsubono Y, Ogawa K, Watanabe Y, Nishino Y, Tsuji I, Watanabe T *et al.* Food frequency questionnaire and a screening test. *Nutr Cancer* 2001; **39**: 78–84.
31 Brown CC, Kipnis V, Freedman LS, Hartman AM, Schatzkin A, Wacholder S. Energy adjustment methods for nutritional epidemiology: the effect of categorization. *Am J Epidemiol* 1994; **139**: 323–338.
32 Willett W. *Nutritional Epidemiology*, 2nd edn. Oxford University Press: New York, 1998.
33 Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol* 1986; **124**: 17–27.
34 Alonso A, de la Fuente C, Martin-Arnau AM, de Irala J, Martinez JA, Martinez-Gonzalez MA. Fruit and vegetable consumption is inversely associated with blood pressure in a Mediterranean population with a high vegetable-fat intake: the Seguimiento Universidad de Navarra (SUN) Study. *Br J Nutr* 2004; **92**: 311–319.
35 Newby PK, Tucker KL. Empirically derived eating patterns using factor or cluster analysis: a review. *Nutr Rev* 2004; **62**: 177–203.
36 Niiranen TJ, Jula AM, Kantola IM, Reunanen A. Comparison of agreement between clinic and home-measured blood pressure in the Finnish population: the Finn-HOME Study. *J Hypertens* 2006; **24**: 1549–1555.
37 Brunner EJ, Thorogood M, Rees K, Hewitt G. Dietary advice for reducing cardiovascular risk. *Cochrane Database Syst Rev* 2005, Art. no. CD002128.
38 Takahashi Y, Sasaki S, Okubo S, Hayashi M, Tsugane S. Blood pressure change in a free-living population-based dietary modification study in Japan. *J Hypertens* 2006; **24**: 451–458.

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研究ノート

小学5年生の学校給食のある日とない日の食事摂取量と食事区分別の比較

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Differences in Food Consumption and Distribution of Meals between the Days with or without School Lunches Among 5th Grade Elementary School Students

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[7]Kagawa Nutrition University

The consumption of nutrients and food groups on days with or without school lunches was assessed among 5th grade elementary school students. The distribution of meals in one day was also compared. A cross-sectional study was conducted from October 2007 to February 2008 in Tokyo and Okayama. Weighing and observation were used to collect data on the school lunches, while dietary records by children accompanied by photographs were used to collect data on meals at home. The study lasted three days, two non-consecutive days with school lunches (weekdays) and one day without (Saturdays or Sundays). The subjects were 82 children with a normal obesity index. The consumption of calcium, vitamin B_1, vegetables, and dairy products was significantly higher on the days with school lunches, while the consumption of salt and seasonings was significantly lower on the days with school lunches. The consumption of calcium, vitamin B_1, and vitamin B_2 at lunch accounted for a larger percentage on the days with school lunches. The results indicate that eating or not eating school lunches affected the consumption of nutrients and food groups for a child's entire day.

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Key words: school lunch, child, dietary survey, meal category

緒　　言

学童期は心身ともに発育が著しい時期である[1)]。学校給食はその回数と質の観点から，学童期の食事において大きな役割を担っている。2007年には，99.2%の小学校において給食（完全給食　97.9%，補食給食　0.5%，ミルク給食　0.8%）が実施されていた[2)]。学校給食の目的の

キーワード：学校給食，児童，食事調査，食事区分別
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1つは，栄養の改善及び健康の増進である。また，児童の望ましい食習慣の形成に寄与することも期待されている[3]。現在，学校給食は，文部科学省より示された「学校給食摂取基準」に基づいて食事計画が行われている。年齢は6－7歳，8－9歳，10－11歳，12－14歳に区分されており，性別による設定はされていない。基準の使用にあたっては，個々の児童生徒の健康及び生活活動等の実態並びに地域の実情に十分配慮し，弾力的に運用することとしている[4]。一日全体としての学童期の食事に関しては，学校給食のある日を対象とした食事内容と体位についての報告がある[5,6]。しかし，学校給食のない日も含めた食事摂取量を検討した報告は少ない[7]。また，「学校給食摂取基準」において，エネルギーの基準は食事摂取基準の値とその他の調査を考慮して求められた男女の一日のエネルギー必要量の平均の1/3を基準にしている[4]。しかし，我が国の食事区分別の摂取状況については，学童期の児童に関する報告[7]，成人についての報告[8]ともに少ない。そこで，本研究では，小学5年生の体格が普通の児童における学校給食のある日（平日）とない日（土曜日または日曜日）の栄養素等と食品群の摂取量及び食事区分別の摂取量について明らかにすることを目的とした。

方　　法

本研究は女子栄養大学倫理審査委員会に研究計画書を提出し，調査実施の承認を得た。児童への調査の説明は，担任がパンフレットを用いて行った。保護者には文書と調査者の連絡先（電話番号等）が渡され，調査についての質問が自由にできるように配慮した。調査の開始にあたり，児童及び保護者より文書にて同意を得た。

対　象　者

東京の2小学校，岡山の3小学校に在籍する小学5年生（10－11歳）317人に調査への参加を依頼し，107名から同意を得た。このうち調査当日に学校を欠席した者1人，身体計測値のない者1人，3日間の調査を完了しなかった者11人を除外した94人（男子38人，女子56人）を解析の対象とした。

調 査 期 間

調査期間は2007年10月から2008年2月の連続しない学校給食のある日2日と学校給食のない日1日の3日間とした。

学校給食の調査

学校給食の調査は調査員による秤量記録法及び観察により実施し，秤量した値の確認のために写真画像を用いた。秤量にはデジタルスケール（タニタ(株)製，No. 1157）を使用した。給食の喫食場所は学校により異なり，教室またはランチルームであった。どちらの場合においても給食の配食は児童により行われた。児童が給食を配食後，調査員が各児童の食事を主食，主菜等の別に計量し，記録した。食事中は調査員が児童の喫食状況を観察した（約10人の児童に対し調査員は1人）。調査員は，児童同士で食事の交換があった場合には，それがだれであったか，またそのおおよその量を記録した。児童がおかわりをした際にも，配食前後に計量し，記録した。喫食後，児童は，調査員が予め児童に渡しておいた番号をトレイや食器と共にテーブルの上に置き，教室またはランチルームを退室した。児童が退室した後，調査員は，各児童の残菜を主食，主菜等の別に計量し，デジタルカメラで撮影，記録した。東京の2校は自校式，岡山の3校はセンター方式により給食が提供されていた。また，岡山の3校の給食は同じセンターにより同じ献立の給食が各学校に提供された。給食は，文部科学省の「学校給食実施基準」（2003年改訂）[9]に基づいた食事計画が行われており，東京都の1校は東京都の「学校給食の栄養摂取基準」[10]に基づいて行われていた。調査は，予め秤量及び児童の観察のトレーニングを受けた調査員により実施された。学校給食の摂取量は各児童の配食量におかわり量を足し，残菜量を差し引いた量とした。

家庭での食事調査

家庭での調査は児童による写真画像を併用した目安量記録法により実施した。学校給食のある日の2日間の食事については，学校において給食の調査を行った日の朝食，夕食，間食について，学校給食のない日は昼食も含めた全ての食事の記録を依頼した。各児童にレンズ付きフィルム（使い捨てカメラ）1台，撮影時の距離を示すリボンを縫い付けたランチマット1枚，食事記録記入用紙3枚，撮影方法を示したパンフレット1部を配布し，食事の記録を依頼した。児童は，喫食前に料理等をランチマットにのせ，使い捨てカメラで食事の内容を撮影し，食事記録記入用紙に食事時間，料理名，食品名，重量または目安量の順に記録した。写真撮影後，食べなかったものがある場合には，料理名または食品名とその分量の記録を依頼した。食事記録用紙には，調査日の各食事の開始時刻及び起床と就寝の時刻の記入も依頼した。また，保護者には，児童による記入漏れがないかの確認を依頼した。本調査に先立ってプレテストを実施した。その後，記録用紙に修正を加え，本調査を実施した。

栄 養 計 算

栄養素等の算出には，栄養計算ソフト「国楽調（バージョン2007）」（NTT）を用いた[11]。五訂増補日本食品標準成分表[12]を使用し，栄養素等及び食品群別の摂取量を算出した。得られた値は強化栄養素及び調理損失を考慮した計算値となっている。日本人の食事摂取基準2005年版（食事摂取基準）[13]の各基準のうち，推定エネルギー

必要量（EER）は男女ともに身体活動レベルIIと各児童の体重を用いて算出した。たんぱく質の推定平均必要量（EAR）と推奨量（RDA）についても各児童の体重を用いて算出した。ビタミンB_1とビタミンB_2のEARとRDAについては各児童のEERを用いて算出した。その他の栄養素については，食事摂取基準に示されている10－11歳の性別のEAR，RDA，目標量（DG）を用いた。児童の食事摂取量を日本人の食事摂取基準の各基準を用いて評価する際には，体格が普通の児童のみを対象にした。

食生活及び生活習慣に関する調査

食生活及び生活習慣に関して，自記式のアンケートを実施した。アンケートの項目は，テレビを見る回数，便通など計19項目とした。女子においては，月経についての項目を加えた。アンケート用紙は教室で配布し，児童が回答した。その後，担任がアンケート用紙を回収した。

身体計測

本調査では，学校保健法（2009年4月に学校保健安全法に改正[14]）により2007年4月に測定された身長と体重の記録を各学校より得た。本研究では，学校保健で用いられている身長別標準体重から判定する肥満度[15]を用いて，体格を評価した。肥満度は次の式で求めた。

肥満度＝（実測体重（kg）－身長別標準体重（kg））／身長別標準体重（kg）×100（%）

肥満度を計算して，これが＋20%以上であれば肥満傾向，－20%以下であればやせ傾向，－20%未満＋20%未満を普通と区分した。

統計解析

データ解析にはSPSS（バージョン15.0）を用いた。学校給食のある日とない日における栄養素等及び食品群の摂取量，食生活の時刻の比較には対応のあるt検定を行った。学校給食のある日とない日における食事区分別の栄養素等の摂取量の割合の比較は対応のあるt検定により行った。群間の差は$p<0.05$のとき有意と判断した。

結　　果

対象者（男子38人，女子56人）の身長の平均は男子141.7 cm，女子140.4 cm，体重の平均は男子35.7 kg，女子33.9 kg，BMIの平均は男子17.7 kg/m^2，女子17.1 kg/m^{2}であった。肥満度の判定によりやせ傾向と判断された者は男女各2人，肥満傾向と判断された者は男女各4人であった。この後の解析については，肥満度の判定により普通と判断された男子32人，女子50人を対象とした。対象者の属性を表1に示した。生活習慣では，テレビを毎日見る者は男子78.1%，女子86.0%であった。男女ともに9割の者が，便通についてほぼ一日一回便通がある，または，比較的よいと回答した。

表2には学校給食のある日とない日における栄養素等の摂取量を男女別に示した。一日の全体の食事として，男女ともカルシウムとビタミンB_1において，学校給食のない日よりもある日に摂取量が有意に高く（男子（カルシウム　ある日：860 mg，ない日：632 mg　$p<0.001$，ビタミンB_1ある日：1.34 mg，ない日：1.01 mg　$p<0.001$），女子（カルシウム　ある日：761 mg，ない日：530 mg　$p<0.001$，ビタミンB_1ある日：1.15 mg，ない日：0.87 mg　$p<0.001$）），食塩においては学校給食のない日よりもある日に摂取量が有意に低かった（男子（ある日：8.8 g，ない日：9.9 g　$p<0.05$），女子（ある日：7.8 g，ない日：10.3 g　$p<0.001$））。食事区分別にみると男女ともに昼食と間食において学校給食のある日とない日の栄養素の摂取量には違いがあった。昼食では，男女ともエネルギー，たんぱく質，炭水化物，カルシウム，鉄，亜鉛，ビタミンA，ビタミンB_1，ビタミンB_2において学校給食のない日よりもある日に摂取量が有意に高かった。食塩においては学校給食のない日よりもある日に摂取量が有意に低く，食事区分別にみると，男女ともに昼食において摂取量が有意に異なっていた（男子（ある日：2.1 g，ない日：3.5 g　$p<0.001$），女子（ある日：1.7 g，ない日：3.6 g　$p<0.001$））。学校給食のある日の昼食のビタミンB_1について，表中の数値は米への強化栄養素（男子：0.29 mg±0.10 mg，女子：0.23 mg±0.08 mg）が含まれた数値である。ビタミンB_1が強化された米は，5校のうち4校で使用されていた。

表3には学校給食のある日とない日における食品群の摂取量を男女別に示した。一日の全体の食事として，男女とも野菜類，乳類において学校給食のない日よりもある日に摂取量が有意に高く（男子（野菜類　ある日：326.0 g，ない日：242.3 g　$p<0.001$，乳類　ある日：411.2 g，ない日：238.0 g　$p<0.001$），女子（野菜類　ある日：274.3 g，ない日：202.5 g　$p<0.001$，乳類　ある日：355.1g，ない日：181.2 g　$p<0.001$）），調味料類においては学校給食のない日よりもある日に摂取量が有意に低かった（男子（ある日：178.5 g，ない日：223.3 g　$p<0.05$），女子（ある日：134.4 g，ない日：172.9 g　$p<0.05$））。昼食では，男女とも豆類，野菜類，緑黄色野菜，淡色野菜，乳類において，学校給食のない日よりもある日に摂取量が有意に高く，嗜好飲料類と調味料類においては学校給食のない日よりもある日に摂取量が有意に低かった。

学校給食のある日とない日における栄養素等の摂取量の食事区分別の割合を男女別に図1-1，1-2に示した。男女ともにエネルギーの摂取量の割合について，夕食の摂取量は学校給食のない日に比べてある日で有意に低く（男子（ある日：29.3%，ない日：34.0%　$p<0.05$），女子（ある日：31.1%，ない日：33.6%　$p<0.05$），

表1 対象者の属性

	男子 $n=32$		女子 $n=50$	
身長 (cm)	141.2 ± 4.9		140.0 ± 5.1	
体重 (kg)	33.8 ± 4.9		32.9 ± 4.5	
BMI (kg/m^2)	16.9 ± 1.7		16.7 ± 1.6	
	n	(%)	n	(%)
月経				
あり	NA		6	(12.0)
なし	NA		44	(88.0)
通学方法				
徒歩のみ	8	(25.0)	18	(36.0)
徒歩＋電車／バス	24	(75.0)	32	(64.0)
テレビを見る回数				
毎日見る	25	(78.1)	43	(86.0)
週に4－5回見る	3	(9.4)	4	(8.0)
週に2－3回見る	1	(3.1)	1	(2.0)
ほとんど見ない	3	(9.4)	2	(4.0)
テレビゲームで遊ぶ回数				
毎日遊ぶ	3	(9.4)	4	(8.0)
週に4－5回遊ぶ	4	(12.5)	4	(8.0)
週に2－3回遊ぶ	14	(43.8)	18	(36.0)
ほとんど遊ばない	11	(34.4)	24	(48.0)
便通				
ほぼ一日一回便通がある	19	(59.4)	25	(50.0)
比較的よい	12	(37.5)	22	(44.0)
どちらかというと便秘気味である	1	(3.1)	1	(2.0)
無回答	−		2	(4.0)
食物によるアレルギーを経験したことがあるか[a)]				
ある	3	(9.4)	4	(8.0)
ない	29	(90.6)	45	(90.0)
無回答	−		1	(2.0)
クラブ活動や学習塾などの習い事に通っているか[b)]				
はい	32	(100.0)	48	(96.0)
いいえ	0	(0.0)	2	(4.0)

平均±標準偏差
NA: Not applicable
a）過去一年の間で
b）学校と地域を含む

間食の摂取量は学校給食のない日に比べてある日では有意に高かった（男子（ある日：13.3%，ない日：9.3% $p<0.05$），女子（ある日：12.8%，ない日：9.9% $p<0.05$））。女子においては，エネルギーの摂取量の割合について，朝食の摂取量が学校給食のない日に比べてある日で有意に低く，昼食の摂取量は学校給食のない日に比べてある日で有意に高かった。男女ともにカルシウム，ビタミン B_1，ビタミン B_2 について学校給食のない日に比べてある日の昼食での摂取量の割合が大きかった。男女ともに学校給食のある日では9割以上の者が間食をしており，朝食と昼食及び起床時刻において，学校給食のある日に比べてない日の時刻は有意に遅かった（表4）。

表5には日本人の食事摂取基準[13]の各基準を用いた学校給食のある日とない日における栄養素等の摂取量の評価を男女別に示した。エネルギーについて，男子において学校給食のない日は75.0%の者がEER未満であり，女子では学校給食のある日とない日ともに50.0%の者がEER未満の摂取量であった。カルシウムの摂取量について，DG未満であった者の割合は，学校給食のある日では男子40.6%，女子68.0%であり，学校給食のない日では男女ともに8割以上であった。鉄の摂取量について，EAR未満であった者の割合は，男子では学校給食のある日56.2%，ない日62.5%であり，女子では学校給食のある日とない日ともに9割以上であった。ビタミン B_1 とビタミン B_2 の摂取量について，学校給食のある日では男女ともにEAR未満であった者の割合は1割未満であり，学

表2　学校給食のある日とない日におけるエネルギー及び栄養素別摂取量（男女別）

			男子（$n=32$）			女子（$n=50$）		
			ある日 a)	ない日 b)		ある日 a)	ない日 b)	
全体	エネルギー	kcal	2,188 ± 274	2,007 ± 477	*	1,920 ± 218	1,853 ± 372	
	たんぱく質	g	82.8 ± 11.3	73.4 ± 20.4	**	73.9 ± 10.0	70.4 ± 17.6	
	脂質	g	71.4 ± 13.8	70.4 ± 31.1		64.2 ± 11.3	63.0 ± 22.3	
	炭水化物	g	296.2 ± 45.9	264.4 ± 46.7	***	255.6 ± 34.5	245.4 ± 49.5	
	カルシウム	mg	860 ± 195.0	632 ± 303.2	***	761 ± 202.9	530 ± 218.5	***
	マグネシウム	mg	259 ± 41.0	233 ± 58.1	**	232 ± 34.9	223 ± 59.3	
	鉄	mg	7.6 ± 1.7	7.5 ± 2.2		7.2 ± 1.7	7.0 ± 2.1	
	亜鉛	mg	9.8 ± 1.2	9.0 ± 2.5		8.6 ± 1.2	8.2 ± 2.3	
	ビタミン A	μgRE	804 ± 357.6	776 ± 734.0		720 ± 313.4	537 ± 327.4	***
	ビタミン B_1	mg	1.34 ± 0.23	1.01 ± 0.47	***	1.15 ± 0.24	0.87 ± 0.26	***
	ビタミン B_2	mg	1.54 ± 0.33	1.36 ± 0.61		1.40 ± 0.31	1.12 ± 0.32	***
	ビタミン C	mg	102 ± 43.4	111 ± 77.1		84 ± 42.5	71 ± 35.3	*
	食物繊維	g	13.4 ± 2.7	12.8 ± 3.2		11.9 ± 2.6	11.7 ± 3.4	
	食塩相当量	g	8.8 ± 1.8	9.9 ± 2.7	*	7.8 ± 1.7	10.3 ± 3.1	***
	脂肪エネルギー比率	%	29.3 ± 3.8	30.5 ± 7.2		29.9 ± 3.5	30.1 ± 6.1	
朝食	エネルギー	kcal	494 ± 117	494 ± 166		450 ± 113	517 ± 144	**
	たんぱく質	g	20.6 ± 5.5	19.3 ± 7.8		17.5 ± 5.8	18.6 ± 6.1	
	脂質	g	17.4 ± 8.1	17.3 ± 12.0		15.1 ± 5.9	18.2 ± 9.6	*
	炭水化物	g	62.9 ± 18.7	65.0 ± 20.1		60.7 ± 15.7	69.5 ± 23.2	**
	カルシウム	mg	235 ± 106.7	253 ± 185.1		197 ± 138.3	208 ± 121.8	
	マグネシウム	mg	67 ± 23.8	67 ± 31.2		56 ± 17.0	59 ± 24.6	
	鉄	mg	1.8 ± 1.1	2.2 ± 1.8		1.9 ± 1.4	2.0 ± 1.2	
	亜鉛	mg	2.3 ± 0.6	2.3 ± 1.0		1.9 ± 0.6	2.0 ± 0.7	
	ビタミン A	μgRE	231 ± 288.6	318 ± 594.5		208 ± 204.9	177 ± 182.5	
	ビタミン B_1	mg	0.27 ± 0.14	0.26 ± 0.19		0.24 ± 0.12	0.26 ± 0.14	
	ビタミン B_2	mg	0.44 ± 0.16	0.50 ± 0.36		0.40 ± 0.24	0.42 ± 0.21	
	ビタミン C	mg	23 ± 13.1	40 ± 69.6		26 ± 22.5	24 ± 19.6	
	食物繊維	g	2.8 ± 1.3	3.3 ± 1.9		2.7 ± 1.1	3.0 ± 1.4	
	食塩相当量	g	2.4 ± 1.0	2.3 ± 1.2		2.1 ± 1.0	2.2 ± 1.1	
	脂肪エネルギー比率	%	30.9 ± 11.0	28.9 ± 13.5		29.3 ± 7.7	30.9 ± 11.0	
昼食	エネルギー	kcal	753 ± 149	638 ± 255	*	618 ± 88	521 ± 169	***
	たんぱく質	g	30.2 ± 6.1	21.9 ± 9.5	***	24.7 ± 4.4	17.5 ± 7.6	***
	脂質	g	22.1 ± 5.4	20.9 ± 13.7		18.3 ± 3.4	15.3 ± 11.7	
	炭水化物	g	104.1 ± 23.3	88.3 ± 31.1	**	85.7 ± 12.8	75.9 ± 24.2	*
	カルシウム	mg	381 ± 69.5	172 ± 141.2	***	339 ± 50.2	115 ± 103.4	***
	マグネシウム	mg	88 ± 22.7	66 ± 30.5	**	73 ± 15.8	53 ± 22.6	***
	鉄	mg	2.6 ± 0.9	2.1 ± 0.9	*	2.0 ± 0.5	1.7 ± 0.7	**
	亜鉛	mg	3.7 ± 0.7	2.4 ± 1.2	***	3.1 ± 0.5	2.0 ± 0.9	***
	ビタミン A	μgRE	309 ± 110.3	193 ± 185.2	**	247 ± 66.2	102 ± 69.7	***
	ビタミン B_1	mg	0.61 ± 0.13	0.36 ± 0.27	***	0.53 ± 0.09	0.23 ± 0.14	***
	ビタミン B_2	mg	0.57 ± 0.07	0.40 ± 0.34	**	0.50 ± 0.06	0.24 ± 0.16	***
	ビタミン C	mg	26 ± 11.4	29 ± 24.4		22 ± 7.2	20 ± 20.5	
	食物繊維	g	4.2 ± 1.7	4.1 ± 1.7		3.4 ± 0.9	3.6 ± 1.7	
	食塩相当量	g	2.1 ± 0.9	3.5 ± 1.8	***	1.7 ± 0.6	3.6 ± 1.6	***
	脂肪エネルギー比率	%	26.4 ± 3.5	27.4 ± 10.4		26.6 ± 2.9	24.2 ± 13.1	
夕食	エネルギー	kcal	636 ± 127	684 ± 231		596 ± 121	617 ± 151	
	たんぱく質	g	25.1 ± 5.4	29.0 ± 13.2		26.2 ± 6.9	30.4 ± 11.5	*
	脂質	g	22.3 ± 6.6	25.5 ± 16.1		21.5 ± 7.7	23.3 ± 13.2	
	炭水化物	g	80.9 ± 20.6	81.4 ± 16.5		71.6 ± 17.0	67.9 ± 18.1	
	カルシウム	mg	146 ± 82.0	160 ± 124.8		143 ± 59.6	149 ± 82.5	
	マグネシウム	mg	81 ± 18.4	88 ± 32.4		82 ± 21.2	96 ± 37.9	**
	鉄	mg	2.5 ± 0.6	2.9 ± 1.5		2.7 ± 0.8	3.0 ± 1.3	
	亜鉛	mg	3.1 ± 0.5	3.8 ± 2.2		3.0 ± 0.8	3.7 ± 1.7	**
	ビタミン A	μgRE	189 ± 86.8	228 ± 295.4		184 ± 106.6	209 ± 214.3	
	ビタミン B_1	mg	0.37 ± 0.15	0.33 ± 0.24		0.30 ± 0.11	0.34 ± 0.18	
	ビタミン B_2	mg	0.37 ± 0.18	0.36 ± 0.19		0.35 ± 0.10	0.38 ± 0.18	
	ビタミン C	mg	35 ± 20.4	35 ± 29.6		26 ± 13.7	22 ± 17.4	
	食物繊維	g	5.0 ± 1.4	4.7 ± 1.9		4.6 ± 1.7	4.4 ± 2.1	
	食塩相当量	g	3.7 ± 1.1	3.8 ± 1.5		3.6 ± 1.1	4.1 ± 2.0	
	脂肪エネルギー比率	%	31.2 ± 6.8	31.4 ± 9.4		31.8 ± 7.8	32.7 ± 11.0	
間食	エネルギー	kcal	306 ± 159	191 ± 174	**	256 ± 161	199 ± 214	
	たんぱく質	g	6.9 ± 4.8	3.2 ± 4.3	**	5.5 ± 4.2	3.9 ± 5.2	*
	脂質	g	9.5 ± 6.4	6.7 ± 9.0		9.4 ± 7.2	6.1 ± 8.8	*
	炭水化物	g	48.3 ± 27.8	29.7 ± 26.2	**	37.6 ± 24.8	32.1 ± 32.6	
	カルシウム	mg	99 ± 92.2	47 ± 78.9	*	82 ± 81.0	58 ± 80.7	*
	マグネシウム	mg	23 ± 13.7	12 ± 12.1	**	21 ± 16.3	14 ± 17.9	*
	鉄	mg	0.6 ± 0.4	0.3 ± 0.4	**	0.6 ± 0.6	0.4 ± 0.6	*
	亜鉛	mg	0.8 ± 0.5	0.5 ± 0.6	*	0.6 ± 0.4	0.5 ± 0.7	
	ビタミン A	μgRE	75 ± 102.4	38 ± 53.8		81 ± 127.6	49 ± 130.5	
	ビタミン B_1	mg	0.09 ± 0.06	0.06 ± 0.08		0.08 ± 0.07	0.04 ± 0.06	**
	ビタミン B_2	mg	0.16 ± 0.15	0.09 ± 0.13		0.16 ± 0.14	0.09 ± 0.12	**
	ビタミン C	mg	19 ± 42.5	7 ± 13.2		11 ± 18.9	4 ± 9.8	*
	食物繊維	g	1.3 ± 0.8	0.7 ± 0.9	**	1.2 ± 1.0	0.7 ± 1.1	*
	食塩相当量	g	0.6 ± 0.4	0.3 ± 0.5	*	0.5 ± 0.4	0.4 ± 0.7	
	脂肪エネルギー比率	%	23.0 ± 11.9	20.2 ± 21.7		26.6 ± 15.5	16.2 ± 18.7	**

平均±標準偏差，　*$p<0.05$　**$p<0.01$　***$p<0.001$，対応のある t 検定

a）学校給食のある日。2日間の平均を用いた

b）学校給食のない日

表3　学校給食のある日とない日における食品群別摂取量（男女別）

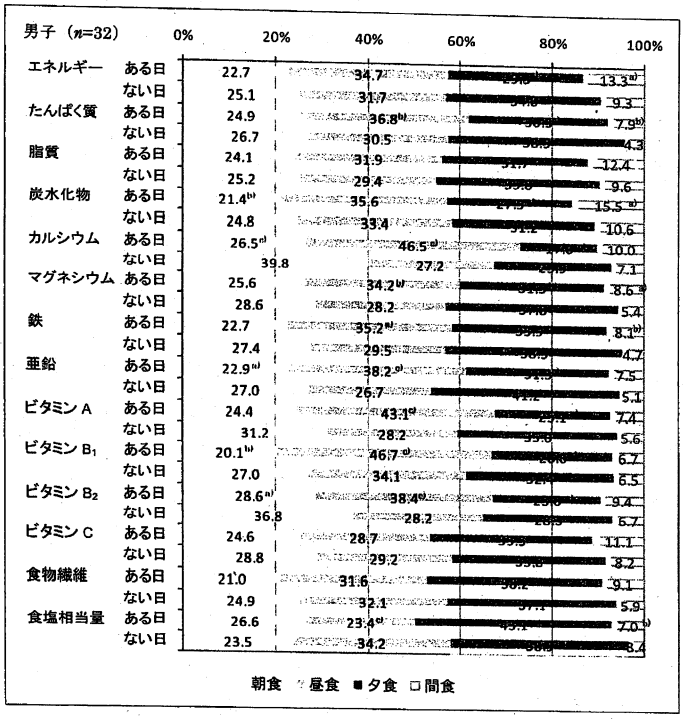
			男子（n＝32）			女子（n＝50）		
			ある日 a)	ない日 b)		ある日 a)	ない日 b)	
全体	穀類	g	469.2 ± 108.3	417.6 ± 101.7	*	392.3 ± 60.6	387.8 ± 106.8	
	芋類	g	45.3 ± 33.7	42.5 ± 40.2		35.4 ± 27.9	30.2 ± 29.8	
	砂糖類	g	6.8 ± 3.7	3.2 ± 3.7	***	6.4 ± 3.6	6.6 ± 8.7	
	豆類	g	51.5 ± 33.1	37.4 ± 41.5		45.8 ± 27.6	43.9 ± 57.0	
	種実類	g	2.2 ± 4.8	1.5 ± 4.7		1.7 ± 2.5	1.9 ± 6.4	
	野菜類 c)	g	326.0 ± 120.0	242.3 ± 123.7	***	274.3 ± 100.6	202.5 ± 105.2	***
	緑黄色野菜	g	120.3 ± 54.7	91.9 ± 51.1	*	104.3 ± 47.2	72.1 ± 52.8	***
	淡色野菜	g	169.8 ± 63.9	115.6 ± 48.5	***	144.5 ± 55.3	106.7 ± 57.2	***
	果実類	g	112.1 ± 69.4	143.2 ± 111.3		114.8 ± 95.2	108.6 ± 96.7	
	きのこ類	g	10.5 ± 7.8	8.9 ± 14.6		13.6 ± 12.9	13.7 ± 20.3	
	海藻類	g	7.9 ± 7.7	9.9 ± 11.5		10.3 ± 10.0	8.6 ± 17.8	
	魚介類	g	50.7 ± 31.1	57.1 ± 53.6		63.1 ± 41.1	70.6 ± 76.8	
	肉類	g	112.4 ± 33.6	116.4 ± 90.7		88.4 ± 34.6	89.4 ± 58.3	
	卵類	g	48.4 ± 24.9	39.4 ± 31.0		43.6 ± 25.5	39.6 ± 31.0	
	乳類	g	411.2 ± 149.9	238.0 ± 213.7	***	355.1 ± 153.0	181.2 ± 148.2	***
	油脂類	g	10.3 ± 5.3	12.5 ± 9.7		9.6 ± 5.0	11.5 ± 8.8	
	菓子類	g	52.1 ± 43.8	39.1 ± 50.7		53.6 ± 47.6	43.2 ± 51.0	
	嗜好飲料類	g	332.5 ± 192.9	429.6 ± 357.0		273.5 ± 255.7	394.2 ± 367.7	*
	調味料類	g	178.5 ± 80.4	223.3 ± 129.9	*	134.4 ± 80.3	172.9 ± 125.5	*
朝食	穀類	g	96.5 ± 47.0	88.8 ± 53.0		80.8 ± 31.0	85.6 ± 63.8	
	芋類	g	5.3 ± 11.5	4.0 ± 20.8		5.3 ± 8.3	4.3 ± 12.5	
	砂糖類	g	0.3 ± 0.7	1.0 ± 2.2		1.4 ± 2.3	1.7 ± 4.5	
	豆類	g	12.4 ± 19.7	12.0 ± 23.0		9.0 ± 17.3	5.6 ± 13.7	
	種実類	g	0.2 ± 1.1	0.4 ± 1.8		0.2 ± 0.6	0.0 ± 0.2	
	野菜類 c)	g	60.4 ± 71.8	57.2 ± 68.3		47.7 ± 50.7	37.1 ± 57.0	
	緑黄色野菜	g	14.4 ± 14.5	16.2 ± 19.5		15.5 ± 22.5	13.1 ± 21.0	
	淡色野菜	g	19.9 ± 18.7	18.7 ± 31.7		12.8 ± 14.2	12.1 ± 21.9	
	果実類	g	41.2 ± 43.1	44.5 ± 42.7		51.5 ± 57.2	54.6 ± 68.8	
	きのこ類	g	0.6 ± 1.6	1.3 ± 3.6		1.2 ± 2.6	1.7 ± 5.9	
	海藻類	g	1.9 ± 3.5	2.4 ± 6.2		1.9 ± 3.7	2.1 ± 5.8	
	魚介類	g	13.8 ± 22.1	6.0 ± 14.8		9.3 ± 20.0	5.0 ± 15.3	
	肉類	g	18.2 ± 20.1	15.3 ± 27.6		13.1 ± 12.9	15.2 ± 20.3	
	卵類	g	18.2 ± 20.0	21.2 ± 22.1		20.8 ± 22.1	20.2 ± 23.3	
	乳類	g	125.6 ± 90.6	129.0 ± 111.7		87.3 ± 90.8	100.9 ± 91.5	
	油脂類	g	1.6 ± 1.6	2.7 ± 3.7		2.1 ± 2.1	2.6 ± 3.6	
	菓子類	g	0.2 ± 1.3	3.2 ± 17.7		11.3 ± 28.3	12.2 ± 32.5	
	嗜好飲料類	g	57.7 ± 81.6	40.6 ± 93.7		57.1 ± 71.0	32.4 ± 63.6	*
	調味料類	g	57.3 ± 61.3	49.9 ± 68.2		44.9 ± 54.5	36.5 ± 61.4	
昼食	穀類	g	214.2 ± 58.3	170.3 ± 78.4	*	172.7 ± 35.7	162.7 ± 63.5	
	芋類	g	3.3 ± 3.4	11.6 ± 21.6	*	3.3 ± 4.6	10.8 ± 23.8	*
	砂糖類	g	3.5 ± 2.2	0.6 ± 1.9	***	2.6 ± 1.3	1.9 ± 6.4	
	豆類	g	20.1 ± 11.8	5.5 ± 15.3	***	15.8 ± 10.5	5.6 ± 18.8	**
	種実類	g	0.9 ± 2.3	0.0 ± 0.2	*	0.6 ± 1.2	1.0 ± 5.1	
	野菜類 c)	g	141.4 ± 80.5	66.9 ± 51.0	***	110.1 ± 49.9	54.9 ± 39.9	***
	緑黄色野菜	g	54.5 ± 36.0	27.5 ± 31.8	**	40.0 ± 17.4	18.2 ± 19.9	***
	淡色野菜	g	86.9 ± 48.7	32.7 ± 27.4	***	70.2 ± 36.3	34.3 ± 32.0	***
	果実類	g	9.7 ± 20.6	45.5 ± 64.0	**	11.3 ± 21.6	25.8 ± 46.0	
	きのこ類	g	4.3 ± 5.8	1.3 ± 4.3	*	3.7 ± 5.3	2.6 ± 9.0	
	海藻類	g	1.1 ± 2.5	2.7 ± 6.2		1.2 ± 2.9	2.3 ± 11.4	
	魚介類	g	15.0 ± 9.7	11.9 ± 25.3		10.7 ± 6.8	12.6 ± 31.4	
	肉類	g	42.6 ± 16.9	31.1 ± 32.6		33.2 ± 9.6	22.5 ± 22.5	**
	卵類	g	19.6 ± 12.2	14.7 ± 20.4		12.7 ± 7.4	5.8 ± 15.0	**
	乳類	g	207.3 ± 3.4	56.2 ± 109.9	***	202.4 ± 25.3	28.7 ± 61.5	***
	油脂類	g	3.4 ± 1.4	5.7 ± 5.7	*	2.5 ± 0.8	3.7 ± 5.0	
	菓子類	g	0.0 ± 0.0	8.8 ± 35.9		0.0 ± 0.0	2.0 ± 14.1	
	嗜好飲料類	g	11.7 ± 27.6	111.9 ± 145.5	**	11.6 ± 25.4	120.0 ± 223.8	***
	調味料類	g	10.5 ± 6.8	59.0 ± 68.9	***	8.2 ± 4.3	54.5 ± 67.9	***
夕食	穀類	g	141.6 ± 51.4	155.0 ± 49.3		128.0 ± 41.5	126.2 ± 51.9	
	芋類	g	32.8 ± 24.2	23.8 ± 26.5		23.8 ± 22.5	14.4 ± 23.2	*
	砂糖類	g	2.1 ± 1.9	1.2 ± 1.7		1.8 ± 1.6	2.4 ± 4.3	
	豆類	g	18.3 ± 19.0	19.9 ± 34.0		20.8 ± 22.2	32.6 ± 52.9	
	種実類	g	0.2 ± 0.4	1.1 ± 4.5		0.7 ± 2.0	0.8 ± 2.5	
	野菜類 c)	g	118.6 ± 40.8	117.2 ± 53.7		112.4 ± 49.5	106.0 ± 57.7	
	緑黄色野菜	g	51.2 ± 41.2	48.2 ± 33.2		48.0 ± 30.2	40.7 ± 35.4	
	淡色野菜	g	63.0 ± 26.1	64.2 ± 40.3		61.5 ± 32.0	60.2 ± 34.7	
	果実類	g	30.1 ± 42.1	27.7 ± 53.1		17.3 ± 30.3	13.8 ± 43.0	
	きのこ類	g	5.5 ± 6.7	6.3 ± 13.1		8.7 ± 10.7	9.3 ± 17.7	
	海藻類	g	4.5 ± 5.8	4.8 ± 8.3		7.2 ± 8.9	3.8 ± 12.6	
	魚介類	g	21.0 ± 19.7	39.2 ± 41.8	*	42.7 ± 34.1	52.9 ± 64.9	
	肉類	g	50.4 ± 20.6	66.3 ± 83.8		40.4 ± 26.5	51.4 ± 47.9	
	卵類	g	10.0 ± 10.7	3.3 ± 8.7	**	8.8 ± 12.3	12.7 ± 22.6	
	乳類	g	25.2 ± 55.1	27.4 ± 97.0		21.0 ± 46.0	14.3 ± 42.9	
	油脂類	g	4.8 ± 4.4	3.8 ± 6.0		4.6 ± 3.9	5.0 ± 5.9	
	菓子類	g	5.0 ± 16.1	0.0 ± 0.0		4.0 ± 11.7	0.2 ± 1.4	*
	嗜好飲料類	g	112.7 ± 106.6	93.4 ± 122.7		103.1 ± 90.7	109.0 ± 111.6	
	調味料類	g	110.5 ± 56.7	114.4 ± 72.5		80.8 ± 63.1	81.9 ± 78.9	
間食	穀類	g	16.9 ± 26.2	3.4 ± 19.4	*	10.9 ± 23.6	13.2 ± 40.9	
	芋類	g	4.0 ± 16.0	3.1 ± 17.7		3.0 ± 12.8	0.6 ± 4.2	
	砂糖類	g	0.9 ± 2.8	0.4 ± 1.8		0.7 ± 2.4	0.6 ± 2.6	
	豆類	g	0.7 ± 3.3	0.0 ± 0.0		0.2 ± 1.1	0.1 ± 0.4	
	種実類	g	0.9 ± 4.4	0.0 ± 0.0		0.2 ± 1.4	0.0 ± 0.0	
	野菜類 c)	g	5.7 ± 21.7	0.9 ± 5.3		4.1 ± 18.5	4.3 ± 28.3	
	緑黄色野菜	g	0.2 ± 0.9	0.0 ± 0.0		0.9 ± 4.2	0.1 ± 0.8	
	淡色野菜	g	0.0 ± 0.0	0.0 ± 0.0		0.1 ± 0.7	0.0 ± 0.0	
	果実類	g	31.1 ± 42.0	25.5 ± 57.0		34.7 ± 64.4	14.4 ± 47.1	**
	きのこ類	g	0.2 ± 0.9	0.0 ± 0.0		0.0 ± 0.0	0.0 ± 0.0	
	海藻類	g	0.4 ± 2.2	0.0 ± 0.0		0.0 ± 0.1	0.3 ± 1.5	
	魚介類	g	0.9 ± 3.4	0.0 ± 0.0		0.4 ± 1.5	0.0 ± 0.0	
	肉類	g	1.2 ± 4.7	3.8 ± 21.2		1.7 ± 6.6	0.3 ± 2.3	
	卵類	g	0.5 ± 2.7	0.2 ± 0.9		1.3 ± 4.1	0.8 ± 5.7	
	乳類	g	53.1 ± 79.5	25.4 ± 61.5		44.3 ± 57.6	37.3 ± 69.8	
	油脂類	g	0.5 ± 1.8	0.3 ± 1.8		0.4 ± 1.4	0.1 ± 0.9	
	菓子類	g	46.9 ± 38.6	27.2 ± 41.3	*	38.3 ± 31.3	28.8 ± 38.0	
	嗜好飲料類	g	150.5 ± 173.1	183.7 ± 266.3		101.7 ± 158.4	132.8 ± 222.7	
	調味料類	g	0.2 ± 0.9	0.0 ± 0.0		0.5 ± 1.8	0.0 ± 0.3	

平均±標準偏差，　*$p<0.05$　**$p<0.01$　***$p<0.001$，対応のある t 検定

a）学校給食のある日。2日間の平均を用いた

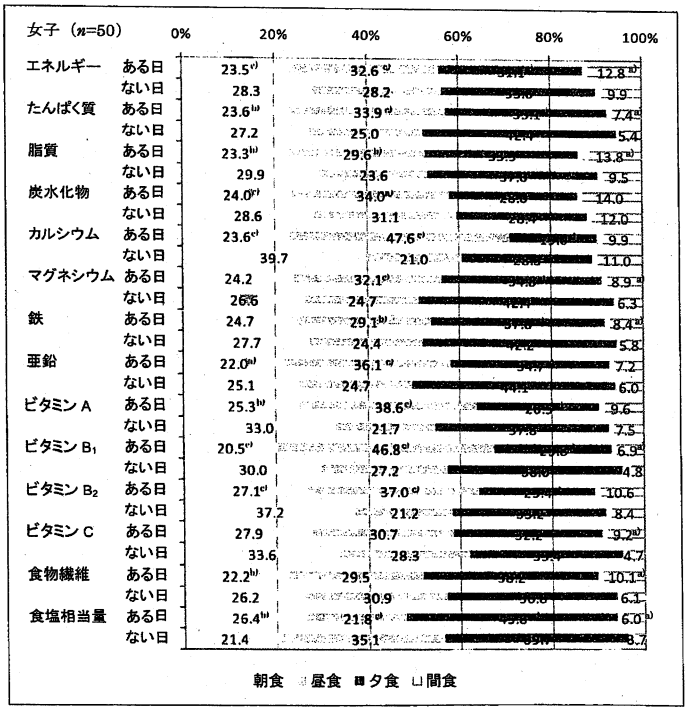
b）学校給食のない日

c）野菜類の内訳は，緑黄色野菜，淡色野菜，漬物である



ある日（学校給食のある日）とない日（学校給食のない日）における各栄養素等について，食事区分別に比較した結果をa）b）c）で表した。a）$p<0.05$，b）$p<0.01$，c）$p<0.001$

図1-1　学校給食のある日とない日における栄養素等摂取量の食事区分別の割合の比較（男子）



ある日（学校給食のある日）とない日（学校給食のない日）における各栄養素等について，食事区分別に比較した結果をa）b）c）で表した。a）$p<0.05$，b）$p<0.01$，c）$p<0.001$

図1-2　学校給食のある日とない日における栄養素等摂取量の食事区分別の割合の比較（女子）

表4 学校給食のある日とない日の食事時刻および起床・就寝時刻と間食の摂取者の割合（男女別）

	男子（$n=32$）			女子（$n=50$）		
	ある日[a]	ない日[b]		ある日[a]	ない日[b]	
朝食時刻 時間：分	6：26 ± 19分	7：33 ± 54分	***	6：37 ± 20分	7：49 ± 55分	***
昼食時刻 時間：分	12：00 ± 0分	12：19 ± 36分	**	12：00 ± 0分	12：24 ± 41分	***
夕食時刻 時間：分	18：57 ± 40分	18：47 ± 43分		18：57 ± 37分	18：48 ± 43分	
起床時刻 時間：分	6：21 ± 16分	7：14 ± 56分	***	6：32 ± 18分	7：36 ± 55分	***
就寝時刻 時間：分	21：48 ± 44分	22：05 ± 54分	**	21：56 ± 102分	22：14 ± 41分	
	n[c] (%)	n (%)		n[c] (%)	n (%)	
間食 あり%	31 (96.9)	22 (68.8)		48 (96.0)	36 (72.0)	

平均±標準偏差， $^{**}p<0.01$ $^{***}p<0.001$，対応のある t 検定
a）学校給食のある日。2日間の平均を用いた
b）学校給食のない日
c）2日間のうち，間食が1日以上あり

表5 食事摂取基準の各基準を用いた学校給食のある日とない日におけるエネルギー及び栄養素別摂取量の評価（男女別）

		男子（$n=32$）		女子（$n=50$）	
		ある日[a]	ない日[b]	ある日[a]	ない日[b]
エネルギー	EER[c] 未満	16 (50.0)	24 (75.0)	25 (50.0)	28 (56.0)
	EER[c] 以上	16 (50.0)	8 (25.0)	25 (50.0)	22 (44.0)
たんぱく質	EAR[c] 未満	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	EAR[c] 以上 RDA[c] 未満	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	RDA[c] 以上	32 (100.0)	32 (100.0)	50 (100.0)	50 (100.0)
カルシウム	DG 未満	13 (40.6)	26 (81.3)	34 (68.0)	44 (88.0)
	DG 以上	19 (59.4)	6 (18.8)	16 (32.0)	6 (12.0)
マグネシウム	EAR 未満	1 (3.1)	5 (15.6)	2 (4.0)	13 (26.0)
	EAR 以上 RDA 未満	2 (6.3)	9 (28.1)	13 (26.0)	10 (20.0)
	RDA 以上	29 (90.6)	18 (56.3)	35 (70.0)	27 (54.0)
鉄（月経なし）	EAR 未満	18 (56.2)	20 (62.5)	16 (32.0)	19 (38.0)
	EAR 以上 RDA 未満	12 (37.5)	8 (25.0)	28 (56.0)	25 (50.0)
	RDA 以上 UL 未満	2 (6.2)	4 (12.5)	6 (12.0)	6 (12.0)
	UL 以上	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
鉄（月経あり）	EAR 未満	NA	NA	46 (92.0)	46 (92.0)
	EAR 以上 RDA 未満	NA	NA	4 (8.0)	3 (6.0)
	RDA 以上 UL 未満	NA	NA	0 (0.0)	1 (2.0)
	UL 以上	NA	NA	0 (0.0)	0 (0.0)
亜鉛	EAR 未満	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	EAR 以上 RDA 未満	3 (9.4)	11 (34.4)	4 (8.0)	8 (16.0)
	RDA 以上	29 (90.6)	18 (56.3)	46 (92.0)	33 (66.0)
ビタミンA	EAR 未満	0 (0.0)	10 (31.3)	0 (0.0)	11 (22.0)
	EAR 以上 RDA 未満	8 (25.0)	6 (18.8)	9 (18.0)	18 (36.0)
	RDA 以上 UL 未満	22 (68.8)	13 (40.6)	40 (80.0)	20 (40.0)
	UL 以上	2 (6.3)	3 (9.4)	1 (2.0)	1 (2.0)
ビタミンB_1	EAR[d] 未満	3 (9.4)	14 (43.8)	2 (4.0)	21 (42.0)
	EAR[d] 以上 RDA[d] 未満	3 (9.4)	7 (21.9)	13 (26.0)	18 (36.0)
	RDA[d] 以上	26 (81.2)	11 (34.4)	35 (70.0)	11 (22.0)
ビタミンB_2	EAR[d] 未満	1 (3.1)	15 (46.9)	2 (4.0)	18 (36.0)
	EAR[d] 以上 RDA[d] 未満	6 (18.8)	4 (12.5)	7 (14.0)	8 (16.0)
	RDA[d] 以上	25 (78.1)	13 (40.6)	41 (82.0)	24 (48.0)
ビタミンC	EAR 未満	9 (28.1)	10 (31.3)	20 (40.0)	29 (58.0)
	EAR 以上 RDA 未満	1 (3.1)	2 (6.3)	8 (16.0)	3 (6.0)
	RDA 以上	22 (68.8)	20 (62.5)	22 (44.0)	18 (36.0)
食塩相当量	DG 未満	20 (62.5)	14 (43.8)	28 (56.0)	12 (24.0)
	DG 以上	12 (37.5)	18 (56.3)	22 (44.0)	38 (76.0)
脂肪エネルギー比率	DG（20%）未満	0 (0.0)	2 (6.3)	0 (0.0)	2 (4.0)
	DG（20%以上30%未満）	20 (62.5)	12 (37.5)	24 (48.0)	23 (46.0)
	DG（30%）以上	12 (37.5)	18 (56.3)	26 (52.0)	25 (50.0)

人数（%），体格が普通の児童のみを対象とした
EER（Estimated Energy Requirment 推定エネルギー必要量），EAR（Estimated Average Requirment 推定平均必要量），RDA（Recommended Dietary Allowance 推奨量），UL（Tolerable Upper Intake Level 上限量），DG（Tentative Dietary Goal for Preventing Life-style Related Diseases 目標量）
NA: Not applicable
a）学校給食のある日。2日間の平均を用いた
b）学校給食のない日
c）各児童の体重に基づき算出した値を用いた
d）各児童の EER に基づき算出した値を用いた

校給食のない日でEAR未満であった者の割合は，男女ともにビタミンB_1では4割，ビタミンB_2では男子46.9%，女子36.0%であった。食塩の摂取量について，DG以上であった者の割合は，学校給食のある日では男子37.5%，女子44.0%であり，学校給食のない日では，男子56.3%，女子76.0%であった。脂肪エネルギー比率がDGの範囲であった者の割合は，男子では学校給食のある日62.5%，ない日37.5%であり，女子では学校給食のある日とない日ともに4割であった。

考　　察

小学5年生の体格が普通の児童において，学校給食のある日とない日の栄養素等及び食品群の摂取量には違いがあることが明らかとなった。この違いを食事区分別にみると，昼食の摂取量において違いがあることが分かる。小学5年生の学校給食のある日とない日の栄養素等の摂取量を調査した報告書によれば，脂質及びビタミンB_1を除く栄養素等の摂取量は，学校給食のない日に比べてある日に高かったとされている[7]。しかし，本研究において，ビタミンB_1の摂取量は，学校給食のない日に比べてある日に高かった。この理由としては，学校給食の米に強化されている栄養素を栄養計算に含んでいることがあげられる。本研究で学校給食のない日よりもある日に摂取量が低かったのは食塩である。食品群別では野菜類と乳類において，学校給食のない日よりもある日に摂取量が高く，食事区分別にみると男女ともに，昼食において摂取量が有意に異なっていた。その結果として，一日全体としての食塩，野菜類や乳類の摂取量の違いにつながったと考えられる。この他，男女ともに昼食での摂取量が一日全体の摂取量に影響したと考えられる栄養素はカルシウムとビタミンB_1である。カルシウムについて，「学校給食実施基準」[9]では，一日の所要量の50%を基準にしており，現在の「学校給食摂取基準」[4]においても食事摂取基準に示されているDGの50%を基準にしている。ビタミンB_1については，「学校給食実施基準」[9]では，一日の所要量の40%を基準にしており，現在の「学校給食摂取基準」[4]においても食事摂取基準に示されているRDAの50%を基準にしている。また，エネルギーの摂取量については，「学校給食実施基準」[9]では，一日の所要量の33%を基準にしており，現在の「学校給食摂取基準」[4]においても食事摂取基準の値とその他の調査を考慮して求められた男女の一日のエネルギー必要量の平均の1/3を基準にしている。従って，エネルギーとほとんどの栄養素が相関する中で，学校給食ではエネルギーに比して，カルシウムとビタミンB_1等を多く摂取できるように献立の工夫がなされているといえる。具体的には，牛乳を毎日提供する，ビタミンB_1が強化されている米を用いるなどである。他国の学校給食の基準をみてみると，英国は日本と同様，学校給食で提供する1日あたりの割合を栄養素によって設定している[16]。例えば，エネルギーはEARの30%，カルシウムはReference Nutrient Intake（RNI: 日本のRDAと同様）の40%などである。しかし，韓国や米国においては，学校給食の基準は全ての栄養素についてRDAの1/3を提供することとしている[17,18]。今後の学校給食摂取基準の改定にあたっては，これら他国の基準やその基準によって提供された学校給食の摂取状況は参考になると考える。また，今回，ビタミンB_1が強化されている米を使用したことで，児童の平日のビタミンB_1の摂取量の分布は高い方向にシフトしたと考えることができる。従って，児童が同じ料理や食品を摂取する給食において，栄養素が強化された食品を用いることは，集団としての栄養素の摂取量の分布のシフトにつながることから，まずは対象とする集団の食物摂取量を把握し，摂取量の分布をどの程度シフトさせることが必要なのかを評価した後に，栄養素が強化された食品を用いることが重要であると考える。

本研究のもう一つの目的である食事摂取量について食事区分別の割合をみると，男女ともに夕食において学校給食のない日に比べてある日のエネルギーの摂取量の割合が低く，間食において学校給食のない日に比べてある日にエネルギーの摂取量の割合が高かった。この理由として，朝食と昼食及び起床時刻が学校給食のある日に比べてない日に遅くなる傾向がみられたことから，朝食，昼食，夕食の間の時間が短くなり，学校給食のない日の間食の摂取量が減少し，夕食の摂取量が増加したのではないかと考えられる。また，食事区分別の摂取量に影響を与えると考えられる学校給食のある日とない日の身体活動量の違いについては，本研究ではその把握が出来なかったため，これに関して検討することはできない。昼食の摂取量については，学校給食のある日の昼食のエネルギー摂取量の割合は男子34.7%，女子32.6%であり，本調査実施時の「学校給食実施基準」[9]の基準（一日の所要量の33%）及び現在の「学校給食摂取基準」[9]（一日のエネルギー必要量の平均の1/3）の基準に近い値であった。従って，学校給食摂取基準のエネルギー摂取量の配分は，体格が普通の児童の実態に即した割合であると考える。次に，間食について，その割合は学校給食のある日（男子13.3%，女子12.8%），ない日（男子9.3%，女子9.9%）であり，先行の報告書（男子10%，女子11%）[7]と同様の結果であった。また，3日間の調査のうち一日以上間食をした者は9割以上であった。これらのことから，学童期の栄養管理において，1割程度の間食を考慮することが現実的であることが示唆された。しかし，間食は他の食事区分に比べてエネルギーに比してビタミン

やミネラルの摂取量が低い傾向にあったことから，間食の内容については検討が必要であると考える。

本調査では，児童の食事摂取量を日本人の食事摂取基準[13]の各基準を用いて評価した。学校給食のある日，ない日ともにEAR未満であった者が多かった栄養素は鉄とビタミンCであった。学校給食のない日よりもある日において，食塩についてDG以上であった者が少なく，ビタミンB_1，ビタミンB_2などの栄養素についてはEAR未満であった者が少なかった。先に述べたように，これらの違いは昼食において生じるところが大きいことから，児童の摂取量に学校給食の果たしている役割が大きいと考えられる。アメリカ栄養士会（American Dietetic Association）は保育施設において，保護者に栄養の情報を提供するために献立を配布することは少ない費用で実行が可能であるとしている[19]。本調査への協力が得られた小学校を含め，多くの日本の学校では，管理栄養士・栄養士が毎月，献立等の情報を保護者に提供している。日本においては，給食における栄養素等の摂取量についての工夫及び献立等の情報を保護者に知らせることをさらに進め，学校給食がない日の食物摂取量を学校給食がある日に近づけることで学童期の食事摂取量の改善に寄与できるものと考える。また，本調査の結果は体格が普通であった児童から得られたものであるが，今後は血圧や生化学検査の結果など，身体計測値以外の指標も組み合わせた食事摂取量の評価が必要であると考えられる。

今回の調査は5つの学校に限定して行ったため，本調査の結果を一般化することには限界がある。しかしながら，本調査の対象児童の体格を学校保健統計の結果（10歳）[20]と比べると，男子の身長と体重の平均は，本調査141.7 cm，35.7 kg，学校保健統計139.0 cm，34.3 kg，女子の身長と体重の平均は，本調査140.4 cm，33.9 kg，学校保健統計140.3 cm，34.3 kgであり，本調査の対象児童が特異な集団とはいえない。本調査の限界として，家庭における食事について，対象者の負担を軽減させるため，学校給食の調査と同様の秤量記録法を用いることができず，写真画像を併用した目安量記録法によって行ったことがあげられる。また，学校給食のある日の調査が2日間であるのに対し，学校給食のない日の調査は1日と調査日数が異なった。食事調査の測定誤差で特に留意を要することの1つとして，日間変動がある[13,21]。本調査では，調査日数が短いために，習慣的な摂取量の分布曲線に比べて，調査から得られた分布曲線の幅は広くなり，摂取不足や過剰摂取を示す者の割合が，習慣的な摂取量から算出する場合に比べて高くなっている可能性は否定できない。今後，学校給食のある日とない日の習慣的な摂取量を評価するにあたり，学校給食のない日についても複数日の調査を実施することが必要であり，今後の課題と考える。

ま と め

学校給食のない日に比べてある日では，カルシウム，ビタミンB_1，野菜類や乳類の摂取量が多かった。この違いは昼食の摂取量によるところが大きく，学校給食のあり，なしが，児童の一日全体の栄養素等及び食品群の摂取量に違いを与えていることが示唆された。食事区分別のエネルギー摂取量の比較について，間食の摂取量は一日の食事の1割程度であり，昼食の摂取量については学校給食摂取基準[9]のエネルギー摂取量の配分に近い値であった。

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文 献

1) World Health Organization: Physical status: the use and interpretation of anthropometry. Technical Report Series No. 854, pp. 263–311 (1995) World Health Organization, Geneva
2) 文部科学省：学校給食実施状況調査，http://www.mext.go.jp/b_menu/toukei/001/kyusyoku/1244845.htm，(2009年11月10日)
3) 文部科学省：学校給食法（1954）
4) 学校給食における児童生徒の食事摂取基準策定に関する調査研究協力者会議：学校給食における食事摂取基準等について（報告）（2008）
5) 齊藤 憲，立身政信：肥満児童の自己記録による食事調査と体重変動の検討，栄養学雑誌，**54**(6), 369–376 (1996)
6) 山本由喜子：小学生の体位と生活活動及び食事内容の関連性，栄養学雑誌，**63**, 235–240 (2005)
7) 独立行政法人日本スポーツ振興センター：学校安全部食の安全課．平成19年度児童生徒の食事状況等調査報告書（2009）
8) 高橋孝子，冨澤真美，伊藤公江，森野眞由美，上西一弘，石田裕美：首都圏在住の既婚勤労男性の1日のエネルギー摂取量の配分の実態，日本栄養・食糧学会誌，**61**, 273–283 (2008)
9) 学校給食における所要栄養量の基準等に関する調査研究協力者会議：学校給食における栄養所要量の基準等について（報告）（2003）
10) 東京都：東京都における児童・生徒一人1回当たりの平均栄養所要量
11) 株式会社NTTデータ：国民健康・栄養調査方式システム国楽調（2003）
12) 文部科学省科学技術・学術審議会資源調査分科会報告：五訂増補日本食品標準成分表（2005）国立印刷局，

東京
13) 第一出版編集部編：日本人の食事摂取基準（2005年版）（2005）第一出版，東京
14) 文部科学省：学校保健安全法（2009）
15) 文部科学省スポーツ・青少年局学校健康教育課 監修：児童生徒の健康診断マニュアル（改訂版）（2008）財団法人日本学校保健会，東京
16) Crawley, H.: Eating well at school: Nutritional and practical guidelines (2005) The Caroline Walker Trust, London
17) Ministry of Education, Science and Technology: Regulation of School Lunch Law in Korea, 2009, http://www.law.go.kr/LSW/Main.html (in Korean), (2009年3月31日)
18) Stallings, V.A. and Taylor, C.L.: Nutrition Standards and Meal Requirements for National School Lunch and Breakfast Programs: Phase I. Proposed Approach for Recommending Revisions (2008) The National Academies Press, Washington, DC
19) American Dietetic Association: Position of the American Dietetic Association: Benchmarks for Nutrition Programs in Child Care Settings, **105**, 979–986 (2005)
20) 文部科学省：学校保健統計調査結果報告（2007）
21) Nelson, M., Black, A.E., Morris, J.A. and Cole, T.J.: Between- and within-subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision, *Am. J. Clin. Nutr.*, **50**, 155–167 (1989)

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事例報告

事業所給食施設におけるヘルシーメニューの給与エネルギー量

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[2)] ソニー健康保険組合

Energy Level of a Healthy Menu Provided at a Worksite Cafeteria

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The energy level was determined of a healthy menu provided in the cafeteria of a worksite aiming at the workers' weight control. The lunch selection in the cafeteria and physical status of the workers, whose BMI was 24 kg/m^2 or higher and who had participated in a nutrition education program for the purpose of losing weight, were examined. The workers had been classified into the Weight Reduction Group or Weight Maintenance/Gain Group by their weight change during the program, and the energy level and nutrient content of the meals selected were compared between the two groups. The average energy levels of the selected meals were 676 ± 73 kcal and 709 ± 64 kcal for the Weight Reduction Group and Weight Maintenance/Gain Group, respectively, the difference being significant. Protein, fat, and the fat energy ratio were also significantly lower for the Weight Reduction Group than for the Weight Maintenance/Gain Group. The distribution of the energy provided by the selected meals examined in 100-kcal intervals shows that the meals selected by the Weight Reduction Group were most frequently in the range of 600–700 kcal, while those by the Weight Maintenance/Gain Group were in the 700–800 kcal range.

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Key words: worksite cafeteria, worker, energy level, healthy menu

緒　　言

勤労者は成人期の健康づくりの推進上，重要な対象者層であり，生活習慣病の一次予防の観点からもその対策は急務である。生活習慣病予防の柱の一つである食生活を通した健康づくりとして，食事を給食や外食に依存している勤労者においては，社員食堂が重要な役割を担っていると考えられる[1)]。社員食堂の給食形態を見ると，2000年には40%を超える事業所がカフェテリア方式を導入し，2007年には約47%と増加傾向にある[2)]。このような変化は顧客満足度を重視した個人の多様なニーズに合わせようとする1つの経営戦略である。しかし同時に，対象者にとっての食環境を整備するという視点から，利用者が適切な食物にアクセスできるようなメニューの提供，モデル献立の提示，栄養成分表示等の見直しが求められている。

社員食堂で提供されているいわゆるヘルシーメニュー（以下ヘルシーメニューとする）のエネルギー量は，72事業所の結果として平均620 kcal（230 kcal－900 kcal）と報告されている[3)]。エネルギー量の設定根拠は不明であり，また，これらの食事の効果は示されていない。そこで本研究は，勤労男性の減量を目標とした社員食堂のヘルシーメニューのエネルギー給与量について検討することを目的とし，社員食堂利用者の，継続的な食事選択状況と身体状況の変化を観察した結果を用いて解析を行った。本研究は，香川栄養学園医学倫理委員会の承認を得，対象者に研究の趣旨を説明，同意を得て実施した。

キーワード：事業所給食施設，勤労男性，ヘルシーメニューの給与エネルギー量
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対象および方法

(1) 対　象　者

対象者は某株式会社の神奈川県内にあるA事業所および東京都内にあるB事業所に勤務する男性社員で，BMI 24 kg/m^{2}以上の者を対象に行われたウェルネススクール参加者である。スクール参加者は36名であり，そのうち女性4名と研究への同意が得られなかった5名を除く27名を対象者とした。

ウェルネススクールは体重減少を目標とした6ヶ月間のプログラムで，社員食堂を受託する給食会社の管理栄養士によるカフェテリアでの料理の組み合わせ方などを中心とした食事に関する講義（60分×3回）と保健師による運動の講義（60分×1回）を受け，各自が目標設定を決め取り組むものである。各自で1ヶ月に減らす体重を設定し6ヵ月後の目標を立て，それを目指すための1日のエネルギー摂取量の設定を行った。昼食は各自が設定した1日のエネルギー摂取量の35%を目標値とした。また対象者はFeliCaシステムに登録することで，食堂での昼食選択状況のセルフモニタリングが可能となっている。

FeliCaシステムは，非接触ICカードFeliCaを用いたデータ自動収集システムである。これは，社員食堂の支払い清算システムで利用されているプリペイド型電子マネーに搭載されている識別IDを利用し，個別に購買履歴を集計するものである。このシステムの利点は，利用者の手を煩わせることなく喫食履歴の情報（料理名及び料理ごとのエネルギー，三大栄養素，食物繊維，食塩相当量，1食の合計値，PFC比率）の収集と蓄積を完全に自動で行うことができ，それによって継続的な昼食の選択状況の観察ができることである。

(2) 調査期間および調査内容

調査はウェルネススクールによる介入期間である2007年5月から11月の6ヵ月間である。昼食選択状況は，FeliCaシステムを用いて，調査期間中6ヵ月間観察した。さらに，同期間に提供された社員食堂の料理のエネルギー量を解析した。また，保健師により，介入前（スクール第1回目開講前），3ヵ月後，6ヵ月後に身体測定（身長，体重，体組成，血圧，腹囲）および血圧測定を行った。

活動量を確認するため，介入前10日間と介入後から3ヵ月までの間，メモリー機能を持つ加速度計付き歩数計（生活習慣記録機 Lifecorder PLUS：株式会社スズケン）を装着してもらった。解析にあたり，加速度計から得られた総消費量から基礎代謝量，身体活動レベル（PAL）を算出した。

(3) 統計解析

体重の減少・維持増加の2群間の平均値の差の検定は対応のないt検定，Mann-Whitney U検定，3群間以上の平均値の差の検定は繰り返しのある一元配置分散分析後，有意差が認められたものについてはBonferroniの多重比較を行った。有意水準は0.05とし，表中の数値は平均値±標準偏差で表した。統計学的処理は統計ソフトSPSS（ver. 11.0 SPSS Inc.）を用いた。

結　　果

(1) 各食堂の概要

それぞれの食堂の概要を表1に示す。いずれも給食の運営は全面委託しており，カフェテリア方式で食事が提供されている。

表1　対象施設の概要

	A事業所		B事業所	
業務	技術開発		事務	
勤務時間	フレックス		フレックス	
従業員数（人）	6,300		4,100	
食堂の運営	委託		委託	
対象食堂	1	2	3	4
受託業者	a	b	a	c
提供方式	カフェテリア		カフェテリア	
昼食の提供料理種類数	22	24	50	50
予定利用者数（人）	2,161	1,700	1,858	1,516

A事業所は社員食堂が4つ設置されており，運営は2社の給食会社（a，b）に2ヵ所ずつ委託している。2社が受託しているうち，各1ヵ所，計2ヵ所の施設を対象とした。両食堂において，小鉢①は70 kcal以下，小鉢②は71 kcal以上の2区分にして提供している。また，b社受託の食堂では主食の大盛りが2－3品提供されている。

B事業所では社員食堂が2つ設置されており，運営は2社の給食会社（a，c）に委託している。その2ヵ所の施設を対象とした。それぞれの食堂において，量を調節している料理（スモールサイズ）が4－5品ある。

すべての食堂において，プライスカードにエネルギー，たんぱく質，食塩相当量の記載がされている。また，Web上で1週間分のメニューおよびエネルギー量を確認することができる。また，対象者はFeliCaシステムによって，選択した食事の栄養成分の履歴を閲覧することができる。

(2) 社員食堂の提供メニューの特性

対象施設の介入期間中（6ヵ月）の提供メニューのうち，主菜のサイズ別のエネルギー量と副菜のエネルギー量を表2に示す。なお，本研究における主菜，副菜の区分は，各食堂で主菜，副菜として提供されていた料理を指す。

レギュラーサイズの主菜の平均エネルギー量は374±

表2　介入期間中に提供された料理のエネルギー量

(kcal)

	平均値±SD
主　菜 レギュラーサイズ	n=2,519 374 ± 140 (99－959)
主　菜 スモールサイズ	n=1,170 259 ± 87 (70－651)
副　菜	n= 804 90 ± 49 (4－339)

( ) は提供エネルギーの範囲

140 kcal であり，99 kcal－959 kcal までの料理が提供されていた。また，100 kcal 単位で分布を確認したところ，300 kcal 以上 400 kcal 未満の料理が最も多く提供されていた。スモールサイズの主菜の平均エネルギー量は 259 ±87 kcal であり，70 kcal－651 kcal までの料理が提供されていた。スモールサイズの主菜では，100 kcal 以上 200 kcal 未満の料理が最も多く提供されていた。また，スモールサイズで，レギュラーサイズと同じような 600 kcal 以上 700 kcal 未満の料理が提供されていた。副菜の平均エネルギー量は 90±49 kcal であり，4 kcal－339 kcal までの料理が提供されていた。副菜は 50 kcal 単位で分布を確認したところ，50 kcal 以上 100 kcal 未満の料理が最も多く提供されていた。副菜として 300 kcal を超える料理も提供されており，その中にはコロッケ・フライ等の揚げ物，脂質の多いサラダがあり，日替わりで毎日提供されていた。

(3) 対象者の特性

1）社員食堂の利用状況と解析対象者

対象者27名のうち，継続的に社員食堂を利用していた者は19名であった。夕食のみの利用，単品のみの利用，自宅からお弁当を持参している者は「食堂利用なし」とした。観察期間中の食堂の営業日数は両事業所とも112日であり，「食堂利用者」の平均食堂利用率は69.5%，「食堂利用なし」の者では17.3%であった。

解析にあたり「食堂利用者」について6ヵ月後（スクール終了時）に体重減少が見られた者と維持または増加した者との間で，昼食の選択状況を比較した。なお，6ヵ月後の身体測定結果がない1名を除き，最終の解析対象者は18名となった。解析対象者を体重減少群，体重維持・増加群の2群にわけ，体重減少群11名，体重維持・増加群は7名となった。平均食堂利用率は体重減少群で72.2%，体重維持・増加群で64.8%であった。

2）身体状況

対象者の身体状況について表3に示す。介入前の身長，体重に両群に有意な差は認められなかった。体重減少群では介入前と3ヵ月後で体重，BMI，腹囲，体脂肪率（p<0.001）が有意に減少した。また，3ヵ月後から6ヵ月後では有意差は認められなかったものの，若干の減少が見られた。体重維持・増加群では，体重，BMI で介入前から3ヵ月後ではほぼ横ばいで推移したが，3ヵ月後から6ヵ月後で有意に増加していた（それぞれ3ヵ月後と6ヵ月後 p<0.01）。また，体脂肪率は介入前から3ヵ月後には有意に減少したものの，6ヵ月後には3ヵ月後より有意に高くなっていた（p<0.01）。

3）活動量

平均総エネルギー消費量，平均歩数，平均 PAL はいずれも体重減少群，体重維持・増加群で有意な差は認められなかった（表4）。

表3　体重変化別の介入期間中の身体状況の変化

	体重減少群　n=11			repeated-measure ANOVA	体重維持・増加群　n=7			repeated-measure ANOVA
	介入前	3ヵ月後	6ヵ月後	p 値	介入前	3ヵ月後	6ヵ月後	p 値
年齢	39.5 ± 7.0				37.1 ± 6.9			
身長（cm）	168.3 ± 5.2				172.5 ± 9.5			
体重（kg）	74.6 ± 10.7[a]	71.1 ± 10.2[b]	70.6 ± 10.3[b]	<0.001	79.9 ± 9.7	79.2 ± 9.3[a]	80.2 ± 9.6[b]	0.003
BMI（kg/m^2）	26.3 ± 2.8[a]	25.0 ± 2.6[b]	24.8 ± 2.6[b]	<0.001	26.8 ± 2.7	26.6 ± 2.6[a]	27.0 ± 2.7[b]	0.004
体脂肪率（%）	23.2 ± 3.5[a]	21.2 ± 3.9[b]	21.0 ± 3.8[b]	<0.001	23.0 ± 2.9[b]	21.8 ± 2.5[a]	23.8 ± 2.2[b]	0.002[1)]
腹囲（cm）	91.3 ± 6.9[a]	86.3 ± 7.3[b]	85.9 ± 7.7[b]	<0.001	93.4 ± 12.6	91.4 ± 8.4	92.1 ± 8.9	0.037
収縮期血圧（mmHg）	127 ± 12	122 ± 7	125 ± 15	0.415	126 ± 13	128 ± 8	133 ± 11	0.195
拡張期血圧（mmHg）	79 ± 5	79 ± 6	78 ± 8	0.746	86 ± 9	81 ± 6	83 ± 7	0.346

[1)] n=6

repeated-measureANOVA　介入前，3ヵ月後，6ヵ月後の3群間での比較

異なる上付きの符号間で有意差あり（Bonferroni 多重比較：p<0.05）

値は平均値±SD

表4 体重変化別の介入後3ヵ月間の活動量

	体重減少群	体重維持・増加群	対応のない t 検定
	$n=11$	$n=7$	p 値
総消費量（kcal）	2,332 ± 191	2,415 ± 264	0.488
歩数（歩）	10,603 ± 2,180	9,408 ± 2,537	0.216
PAL	1.50 ± 0.06	1.47 ± 0.10	0.422

値は平均値±SD

表5 体重変化別の給食でのエネルギーおよび栄養素選択量

	体重減少群	体重維持・増加群	Mann-Whitney U 検定
	$n=11$	$n=7$	p 値
エネルギー（kcal）	676 ± 73	709 ± 64	0.011
たんぱく質（g）	27.4 ± 3.0	29.5 ± 3.3	0.002
脂質（g）	19.7 ± 4.5	23.4 ± 5.0	<0.001
炭水化物（g）	93.9 ± 10.0	91.6 ± 9.4	0.276
食物繊維（g）	5.9 ± 1.2	5.8 ± 1.6	0.643
ナトリウム（食塩相当量）（g）	4.0 ± 0.9	4.3 ± 1.2	0.038
たんぱく質エネルギー比率（%）	16.4 ± 1.4	16.8 ± 1.8	0.358
脂質エネルギー比率（%）	25.1 ± 3.5	28.4 ± 4.3	<0.001
炭水化物エネルギー比率（%）	56.5 ± 4.0	52.8 ± 4.6	<0.001
食堂利用率（%）[1]	72.2 ± 10.9	64.8 ± 15.8	0.25

[1] 食堂利用率＝昼食利用回数／営業日数×100
値は平均値±SD

(4) 食事選択状況

1）6ヶ月間のエネルギーおよび栄養素選択量

介入期間中の給食（昼食）選択状況を表5に示す。体重減少群の平均エネルギー選択量が676±73 kcalであったのに対し，体重維持・増加群では709±64 kcalと有意に多かった（$p<0.05$）。なお，介入前2週間の平均選択量では800 kcal以上の選択であった者も見られたが，介入中は500 kcal以上800 kcal未満の範囲の選択であった。介入中の最頻値は体重減少群で600 kcal以上700 kcal未満，体重維持・増加群では700 kcal以上800 kcal未満であった。

また，たんぱく質，脂質，脂質エネルギー比率，食塩相当量も体重維持・増加群で有意に多かった。炭水化物エネルギー比率は体重減少群で有意に高かった（$p<0.001$）。

考　　察

食堂利用者を体重減少群と体重維持・増加群に分けて給食（昼食）で選択したエネルギー量の違いを検討したところ，体重減少群の平均エネルギー選択量は676±73 kcalであり，体重維持・増加群の709±64 kcalより有意に少なかった。また，選択エネルギー量の分布を見ると，最頻値は体重減少群で600 kcal以上700 kcal未満，体重維持・増加群では700 kcal以上800 kcal未満であった。これらのことから，適正体重を目標に減量の必要がある場合において600－700 kcal程度の昼食を継続的に摂取することは効果がある可能性があると考える。また，体重減少群の脂質エネルギー比率は25.1±3.5%と目標量に近く，また，体重維持・増加群の28.4±4.3%よりも有意に低かった。食物から摂取する脂肪の量が増加すると体重増加につながることは明らかであり[4] 大幅な減量を長期間維持している者はそうでない者よりも脂質エネルギー比率が低いことを示す報告[5] もある。このことから，エネルギー給与量のほかに脂質給与量の設定も重要であり，脂質エネルギー比率の目標量である20%以上25%未満の基準を1食給食においても目標とすることが望ましいと言える。

今回，対象者の身体活動量の変化の有無を継続的にモニタリングするために加速度計法を用いた。対象者の状況から，簡便な方法であることが重要な要素であった。実際の栄養管理業務としての活用を考えれば費用的な面も考慮しなければならない。加速度計法は，エネルギー代謝測定室や二重標識水法を妥当基準とすると誤差を有するとされているが，日常のおおよその身体活動を評価するには妥当であるという報告が多く，歩行，走行を中心とした日常生活・職場での身体活動による消費エネル

ギーの推定に利用できるとされている[6~10]。今回の対象者の身体活動量は3ヶ月目までは変化がなく，また，体重減少群と体重維持・増加群にも違いは認められなかった。

本研究の対象者はPALが1.48でありレベルⅠ（低い）と推定された。この活動量の推定から目標のエネルギー摂取量を設定し，この値に対する昼食の選択量の比率を検討した。体重減少群と体重維持・増加群では推定エネルギー必要量（EER）に占める昼食の割合に有意差は認められず，体重減少群で31.0±3.2%，体重維持・増加群で31.9±3.4%であった。しかし，体重減少が見られた者の昼食のエネルギー量は700 kcal以下であり，維持・増加群よりも有意に少なかった。今回は昼食以外の食事内容を把握していないため，1日の総エネルギー摂取量から食事ごとのエネルギーの配分比率を算出することはできなかった。1日の総エネルギー摂取量の把握ができない条件下で体重減少を目標として昼食のエネルギー量を設定する場合，EERに占める割合で示すよりも600－700 kcal程度として設定した方が適切かもしれない。また，日本人の食事摂取基準のエネルギーの策定において，成人における推定エネルギー必要量の推定誤差は1日当たり概ね±200 kcal－最大300 kcal程度と考えられている。従って，給食の献立作成基準として，推定エネルギー必要量の付近を目指すこととし，幅を設けてエネルギー給与量を設定する必要があると考える。その点から考えると，エネルギー給与量の計画を立てる上で，600－700 kcal程度と幅を持たせ，かつその代表値として650 kcalという基準を設定することが給食の献立計画としては現実的と考える。主菜，副菜を選択して650 kcal程度に抑えるためには，食堂で提供されているごはんを主食として選択すると想定すると302 kcalである（主食からとれる穀物エネルギー比率46%）。従って主菜は200 kcal－300 kcal，副菜は50 kcal－150 kcalで提供されることが望ましい。本研究の対象施設で提供されている主菜の最頻値は315 kcalであり，スモールサイズの主菜の提供もあることから，比較的組み合わせやすいといえる。しかし，1品で600 kcalを超える主菜が提供されていたことは食環境を整備する点からの課題である。減量を目標としたヘルシーメニューを提供する場合，利用者が継続的に望ましい食事を選択できるような食環境の整備が必要と考えられる。

カフェテリア方式による食事の提供は，お客様の満足度も考慮することが経営的に求められるため，嗜好性を満足させるために料理の多様性が必要である。しかし，健康増進法において「献立の作成にあたり，喫食者の給与栄養量が確保できるよう，施設における献立作成基準を作成するよう努めること」とされており，献立作成基準として，提供する料理区分ごとの種類数とそれに応じたおよそのエネルギーおよび栄養素量の範囲が示されることが望まれている[11]。利用者の栄養管理を目指し，エネルギーの適正範囲を献立作成基準として作成しておくことは必要である。また，副菜については，毎日コロッケのような揚げ物が提供されていたり，200 kcalを超える副菜が提供されていた。脂質エネルギー比率を25%程度の適正な範囲に保つためには，主菜のみならず副菜への配慮も必要である。

Heymsfieldらは体重減少がうまくいかない理由として，被験者が指示通りできていないことを挙げており[12]，森らはDHAや大豆イソフラボンを添加したお弁当の提供による効果ではあるが，1日1食でもバランスの良い食事をすることが生活習慣病の予備群に効果があることを支持している[13]。カフェテリア方式は自分で選択できるところに利点がある。多種類多品目が提供されているため，食事内容が多様で豊かになる反面，個人の嗜好に頼った選択がなされていれば健康管理上問題になる[14, 15]。カフェテリア方式による提供においても体重管理のための主食，主菜，副菜がそろう食事を毎日1種類でも提供することが望まれる。

また，有意差は見られなかったが，体重減少群の食堂利用率が72.2%であったのに対し，体重維持・増加群では64.8%であり，体重減少群の方が食堂利用率は高かった。Roosらは，健康的な食事が提供される場合，社員食堂で昼食を食べることが食事の質に関係し，社員食堂を利用している者はそうでない者よりもBMIが低い傾向が見られたとしている。さらに，労働時間中に野菜を含む料理を提供することは，勤労者の食事を改善する効率的な方法かもしれないとしており，社員食堂で昼食を食べる機会を増加させることに重点を置くべきとしている[16]。また，健康増進法施行規則第9条に規定される栄養管理基準を実施している項目が多い施設で給食提供を受けることは，昼食1食であっても利用者の健康や食事について良好な態度形成につながる可能性を示唆する報告もある[17]。健康的な食事にアクセスできることを前提としたときに，社員食堂の利用が健康管理につながると考えられるため，社員食堂の利用を促す手立てが勤労者の健康管理の一つになることが考えられる。

本研究の限界は，1企業の男性従業員を対象とした事例的検討の結果であることである。また，社員食堂における昼食の選択状況データを収集するにあたり利用したシステム上，摂取量ではなく提供栄養量からの検討であるため，4ヵ所の食堂で提供されたメニューの違いによる影響を否定できない。さらに，体重減少については，対象者に同一内容の介入をしていることから，昼食以外の食事を含め，望ましい食事選択のために必要なスキル

や知識が高まり，全体的には望ましい結果が得られている可能性があることである。従って，今後，介入研究を含めた研究の積み重ねが必要である。

ま と め

本研究は，特定給食施設である社員食堂において，適切な栄養管理を行うために，社員食堂利用者の料理選択のアセスメントから得られる情報に基づき，勤労男性の体重管理を目標としたヘルシーメニューのエネルギー給与量の検討を行った。

1）介入期間中6ヵ月の平均エネルギー選択量は体重減少群では676±73 kcal，体重維持・増加群では709±64 kcalであり，体重減少群で有意に少なかった。

2）6ヵ月間の平均脂質エネルギー比率は，体重減少群では25.1±3.5%，体重維持・増加群では28.4±4.3%であり，体重減少群が有意に低かった。

3）昼食の選択エネルギー量の最頻値は体重減少群で600 kcal以上700 kcal未満，体重維持・増加群では700 kcal以上800 kcal未満であった。

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文　　献

1）平澤マキ：「事業所給食」における食育，保健の科学，**48**, 735–739（2006）
2）労務研究所：2007年版　民間企業150事業所対象 職場給食の経営指標と価格，旬刊　福利厚生，**1957**, 5–12（2007）
3）労務研究所：「職場給食の経営指標と価格」付帯調査 社員食堂の栄養，健康管理をみる，旬刊　福利厚生，**1960**, 26–31（2007）
4）Garrow, J.S., James, W.P.T., Ralph, A. 編　渡邊令子訳：ヒューマンニュートリション　第10版，147–158（2004）医歯薬出版，東京
5）Shick, S.M., Wing, R.R., Klem, M.L., McGuire, M.T., Hill, J.O. and Seagle, H.: Persons successful at long-term weight loss and maintenance continue to consume a low calorie, low fat diet, *J. Am. Diet. Assoc.*, **98**, 408–413（1998）
6）田中茂穂：間接熱量測定による1日のエネルギー消費量の評価，体力科学，**55**, 527–532（2006）
7）山田誠二，馬場快彦：運動強度を加味したカロリーカウンターによる運動時消費エネルギー量の測定，産業医科大学雑誌，**12**, 77–82（1990）
8）海老根直之，島田美恵子，田中宏暁，西牟田守，吉武裕，斎藤愼一，Jones, P.J.H.：二重標識水法を用いた簡易エネルギー消費量推定法の評価—生活時間調査法，心拍数法，加速度計法について—，体力科学，**51**, 151–164（2002）
9）横地正裕，新実光朗：糖尿病患者の歩行時にカロリーカウンターによって測定されたエネルギー量の妥当性，理学療法学，**22**, 178–180（1995）
10）横山有見子，川村　孝，玉腰暁子，野田明子，平井真理：加速度計による身体活動量の測定の妥当性，スポーツ医・科学，**12**, 23–27（1999）
11）石田裕美，村山伸子，由田克士：特定給食施設における栄養管理の高度化ガイド・事例集，38–43（2007）第一出版，東京
12）Steven, B.H., Joyce, B.H., Joel, W.B., Dale, A.S., Ngozi, E. and Angelo, P.: Why do obese patients not lose more weight when treated with low-calorie diets? A mechanistic perspective, *Am. J. Clin. Nutr.*, **85**, 346–354（2007）
13）森　真理：ヘルシーランチによる生活習慣病のリスク軽減効果，食品工業，**49**, 40–48（2006）
14）高橋道子，鹿嶋康子，渋谷久恵，鈴木和雄，稲葉佳代子，山本妙子，飯田　稔：カフェテリア方式による事業所給食の実態（第1報）提供されている料理の内容，神奈川県立栄養短期大学紀要，**18**, 53–62（1986）
15）三松永典：社員食堂における栄養管理の実際—栄養展示会—，保健の科学，**42**, 989–993（2000）
16）Eva, R., Sirpa, S.L. and Tea, L.: Having lunch at a staff canteen is associated with recommended food habits, *Public Health Nutr.*, **7**, 53–61（2003）
17）平田亜古（分担研究者）：分担研究報告書　事業所給食における栄養管理の実施状況と利用者の健康と食に対する知識・態度の関係　平成17年度厚生労働科学研究「特定給食施設における栄養管理の実施状況とその基準に関する研究」報告書，87–103（2006）
18）石田裕美：分担研究報告書　従業員食堂を活用したハイリスク者の栄養教育プログラムの検討　平成19年度厚生労働科学研究費補助金　循環器疾患等生活習慣病対策総合研究事業　勤労者の健康づくりのための給食を活用した集団及びハイリスク者への対策に関する研究報告書，45–74（2008）

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Original Article

Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly

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There have been methodological problems for studying hypovitaminosis D and K in the elderly. First, studies were done either by evaluating food intake or measuring their circulating levels, but rarely by both in Japan. In this paper, vitamin D and K intakes and their circulating levels were simultaneously determined. Second issue is whether hypovitaminosis D and K are independent of general malnutrition, prevalent in the elderly. We tried to statistically discriminate them by principal component analysis (PCA). Fifty institutionalized elderly were evaluated for their circulating 25 hydroxy-vitamin D (25OH-D), intact parathyroid hormone (PTH), phylloquinone (PK), menaquinone-7 (MK-7) levels, and their food intake. Although average vitamin D intake (7.0 μg/day) exceeded the Japanese Adequate Intake (AI) of 5.0 μg/day, average serum 25OH-D concentration was in the hypovitaminosis D range (11.1 ng/mL). Median vitamin K intake was 168 μg/day, approximately 2.5 times as high as AI for vitamin K. Nevertheless, plasma PK and MK-7 concentrations were far lower than those of healthy Japanese elderly over 70 years old. PCA yielded four components; each representing overall nutritional, vitamin K_2, vitamin D, and vitamin K_1 status, respectively. Since these components are independent of each other, vitamin D- and K-deficiency in these subjects could not be explained by overall malnutrition alone. In summary, institutionalized elderly had a high prevalence of hypovitaminosis D and K, and the simultaneous determination of their circulating level and dietary intake is mandatory in such studies. PCA would yield fruitful results for eliminating the interference by confounders in a cross-sectional study.

Key Words: hypovitaminosis D, hypovitaminosis K, principal component analysis, adequate intake, institutionalized elderly

INTRODUCTION

Vitamin D is of utmost importance in enhancing the intestinal absorption of calcium and phosphorus,[1,2] with its deficiency causing skeletal mineralization defect; rickets and osteomalacia. Recently, it has come to the general attention that inadequate supply of vitamin D, even in its milder form (vitamin D insufficiency), is associated with increased risk of fracture through negative calcium balance, hence secondary hyperparathyroidism.[1,2] Vitamin D insufficiency is also reported to be associated with muscle weakness. Recent clinical studies have indicated that intervention with vitamin D supplementation reduced the incidence of falling in elderly subjects.[3] Clinically important non-vertebral fractures, such as hip and wrist fractures are triggered by falling. Thus, vitamin D insufficiency would render the elderly subjects more prone to fracture through its effects both on the skeleton and muscle. Recently, lower serum level of 25 hydroxy-vitamin D (25OH-D) was reported to be a significant risk factor even for mortality.[4]

Vitamin D insufficiency is quite common in the elderly population,[5,6] and institutionalized elderly are at even higher risk for vitamin D insufficiency.[7-10] Factors hitherto postulated to be responsible include low dietary vitamin D intake,[7,9] reduced dermal capacity to produce vitamin D with aging and minimal sun exposure.[11,12]

In contrast to vitamin D, the skeletal action of vitamin K has called our attention only quite recently. The only biological action of vitamin K has been considered to be its role as the coenzyme of γ-glutamyl carboxylase (GGCX) in the liver, by which additional carboxyl group is introduced into the glutamic acid residue in four of the

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Table 1. Background profiles and results from blood tests of the study subjects

	Total	Male	Female	*p* value
n	50	15	35	-
Age (y)	87.6±8.0 (88.5)	84.9±7.9 (83.0)	88.7±7.8 (90.0)	0.133
Level of care needed	3.6±1.1 (4.0)	3.3±1.0 (3.0)	3.7±1.2 (4.0)	0.228
Body height (cm)	144.0±11.6 (142.0)	157.0±7.8 (159.0)	138.4±7.8 (139.0)	<0.01
Body weight (kg)	43.6±9.3 (43.2)	50.3±7.9 (49.9)	40.7±8.3 (38.1)	0.001
Body mass index (kg/m2)	21.0±3.8 (20.1)	20.5±3.4 (19.6)	21.3±4.0 (20.2)	0.476
Serum albumin (g/dL)	3.7±0.4 (3.7)	3.8±0.4 (3.9)	3.6±0.4 (3.6)	0.136
Serum total cholesterol (mg/dL)	184±37 (184)	186±26 (195)	183±41 (183)	0.828
Serum triglyceride (mg/dL)	98±41 (92)	96±47 (75)	98±39 (93)	0.403
Serum aminotransferase (U/L)	22±11 (19)	20±7 (17)	22±12 (19)	0.603
Serum alanine aminotransferase (U/L)	16±10 (13)	16±7 (13)	16±12 (12)	0.235
eGFR (mL/min./1.73m2)	61±20 (60)	67±19 (67)	59±21 (57)	0.208
Serum 25-hydroxyvitamin D (ng/mL)	11.1±3.1 (11.2)	10.3±3.5 (9.3)	11.5±3.0 (11.6)	0.274
Serum parathyroid hormone (pg/mL)	30.8±11.8 (30.0)	29.9±11.1 (31.0)	31.3±12.2 (30.0)	0.736
Plasma phylloquinone (ng/mL)	0.73±0.70 (0.58)	0.62±0.29 (0.60)	0.77±0.82 (0.53)	0.992
Plasma menaquinone-7 (ng/mL)	0.53±0.37 (0.45)	0.59±0.47 (0.47)	0.51±0.32 (0.44)	0.849

Data are expressed as mean±SD with the values in parentheses showing the median.
Comparison of indices between males and females were done by unpaired t test or Mann-Whitney test depending on normality.
eGFR; estimated Glomerular Filtration Rate.

Table 2. Daily dietary intakes of the study subjects

	Total	Male	Female	*p* value
Energy (kcal)	1322±159 (1387)	1374±96 (1416)	1300±175 (1386)	0.160
Protein (g)	51.0±5.8 (53.3)	53.1±3.6 (54.6)	50.2±6.3 (53.5)	0.091
Fat (g)	32.8±3.9 (34.6)	34.2±2.4 (35.3)	32.2±4.3 (34.5)	0.095
Carbohydrates (g)	178±20 (186)	185±12 (189.7)	175±21 (186)	0.093
Calcium (mg)	494±53 (504)	503±50 (506)	490±54 (502)	0.157
Vitamin D (μg)	7.0±1.4 (7.7)	7.4±0.9 (7.8)	6.9±1.5 (7.6)	0.107
Vitamin K (μg)	155±30 (168)	164±19 (172)	151±33 (168)	0.107

Data are expressed as mean±SD with the values in parentheses showing the median. Comparison of indices between male and women were done by unpaired t test or Mann-Whitney test depending on normality.

blood coagulation factors (II, VII, IX, X) to yield γ-glutamic carboxyl (Gla) residue.[13] Other extrahepatic proteins are also γ-carboxylated by GGCX, such as osteocalcin (bone Gla protein; BGP) and matrix gla protein (MGP).[14] Recent evidences suggest that vitamin K deficiency is associated with increased risk of fracture. When subjects were categorized into quartiles according to their vitamin K intake, fracture risk in the lowest quartile was twice as high as that in the highest quartile.[15] The age-adjusted incidence of vertebral fracture was significantly higher in subjects with low plasma phylloquinone levels than those with high plasma levels in Japanese women.[16] In addition, the association of circulating vitamin K level and bone mineral density (BMD) has also been reported. For example, low plasma phylloquinone concentration was associated with low BMD at the femoral neck in men, and lower spine BMD in postmenopausal women without estrogen replacements.[17] High serum concentration of undercarboxyled osteocalcin (ucOC), which is a sensitive indicator of skeletal vitamin K insufficiency, was a significant risk factor of hip fracture independent of BMD.[18,19]

Plasma phylloquinone level is subject to alteration by aging,[20,21] and elderly subjects have been reported to have low plasma phylloquinone concentrations.[22] Of note is the report that elderly nursing home residents generally had a poor dietary vitamin K intake compared to the ambulatory elderly.[23]

Studies on the role of hypovitaminosis D and K in the elderly, especially the institutionalized ones are greatly hampered by the fact that they are also generally malnourished. Arguments against the significance of these vitamins have been made that decreased serum concentrations of these vitamins is merely a reflection of overall malnutrition. In this paper, we have tried to statistically discriminate hypovitaminosis D and K from general malnutrition by using principal component analysis (PCA), which has been employed in clinical nutrition for the analyses of dietary pattern.[24,25]

MATERIALS AND METHODS

Subjects

The study subjects were 50 institutionalized elderly (male 15, female 35) in a nursing home, Kayu-Shirakawa. Exclusion criteria were routine medication that has potential interference with vitamin D or vitamin K status. Detailed information about this study was given and written consent was obtained from the subject or the proxy. The study protocol was approved by the ethical committee in Kyoto Women's University.

Laboratory data

Blood was obtained after overnight fasting. After centrifugation, serum was kept frozen at –30°C until analysis. Serum concentration of 25OH-D was measured by radioimmunoassay (RIA) (DiaSorin, Stillwater, MN, USA).

Circulating level of intact parathyroid hormone (PTH) was measured by electro chemiluminescent immunoassay (ECLIA) (Roche Diagnostics, Mannheim, Germany). Plasma vitamin K_1 (phylloquinone; PK), and menaquinone-7 (MK-7) levels were determined by high-performance liquid chromatography-tandem mass-mass spectrometry with atmospheric pressure chemical ionization (LC-APCI-MS/MS) using a HPLC system (Shimadzu, Kyoto, Japan) and API3000 LC-MS/MS System (Applied Biosystems, Foster City, CA) with ^{18}O-labeled vitamin K as the internal standard.[26]

Nutrition intake study

Since the subjects were institutionalized and their diet was supplied from the institution, their nutrients and energy intake were calculated by multiplying the supplied nutrients on the basis of the Standard Tables of Food Composition in Japan, 5th ed. with the average percentage intake in a preceding month by the staff.[27] Percentage intake was assessed for each subject at every meal, and the monthly average percentage intake was calculated. Based on these records, their intake of energy and nutrients was calculated using software (Healthy Maker Pro 501, Mushroom Software Corp, Okayama, Japan).

Statistical analyses

Statistical analyses were performed with SPSS 15.0J (SPSS Japan Inc., Tokyo, Japan). Comparison of two independent groups was made with Student's t-test or Mann-Whitney test depending on normality. Multiple regression analyses by stepwise method were performed to determine independent factors for circulating levels of vitamin D and K levels. The relationship between various nutritional indices and circulating vitamin D- and K- levels was analyzed with principal component analysis (PCA), which is a statistical method to summarize the various parameters into a small number of summary factors (components). These components are obtained in such a way that the first component is extracted from the initial raw data with the maximal amount of information (eigenvalue), and the second one is extracted from the remaining information. Therefore, each component is mutually independent. Components with the eigenvalue greater than 1 were adopted, as in usual practice.

RESULTS

Biochemical markers and circulating concentrations of vitamin D and K

Baseline characteristics and data from blood examination are shown in Table 1. There was no gender difference in the age and level of care needed, which is a 5-grade score in the long-term care insurance in Japan with a higher number indicating the need for more intensive care. The level of care needed was higher than grade 3 in 78% of subjects. Most of the present subjects required wheelchair for transportation. Body height and body weight were significantly higher in males than in females. Body mass index (BMI), or serum albumin, total cholesterol and triglyceride concentrations did not significantly differ between the two groups. Generally, serum albumin level less than 3.5 g/dL is considered to indicate malnutrition. Serum albumin level was below this value in 26% of subjects. Inasmuch as the advanced age and high level of care needed, nutritional parameters remained within the reference range in most of the subjects. None of the study subjects had severe hepatic or renal dysfunction. There is a general consensus that a serum 25OH-D concentration less than 20 ng/mL indicates hypovitaminosis D.[2] Serum 25OH-D concentration was <10 ng/mL in 40% of subjects, 10-20 ng/mL in 58%, and ≥20 ng/mL in only one subject. None of the subjects had a serum PTH level above the cut-off value (65 pg/mL). Plasma PK and MK-7 concentrations in all of the subjects were 0.73±0.70 ng/mL and 0.53±0.37 ng/mL, respectively. In the present study, serum PK was less than 1 ng/ml and serum MK-7 was less than 1 ng/ml, in 85% and 90% of the subjects, respectively. The interpretation for these values will be given in the "Discussion" section. There were no gender differences in plasma vitamin K levels, serum 25OH-D or PTH.

Nutritional intake in the study subjects

The nutrients intake in the males and females were not statistically different as shown in Table 2. During the preparation of this paper, Dietary Reference Intake (DRI) for Japanese 2010 (DRI 2010) was released on May 29, 2009.[28] Since this work was done in 2006, however, consideration is made basically according to DRI 2005.[29] The intake of macronutrients such as protein, fat and carbohydrates appeared appropriate for their age and sex. The adequate intakes (AI) for calcium in Japan are 750 mg for men and 650 mg for women over 70 years. The AI for vitamin D is 5 μg/day, and that for vitamin K is 75 μg/day for men and 65 μg/day for women respectively. Although average calcium intakes in both groups were lower than the AI in DRI 2005, the average daily vitamin D intake was 7.0 μg, which is 140% of the AI in DRI 2005. The average daily intake of vitamin K in whole subjects was 155 μg, which is more than twice the AI for each gender. Thus, apparently these subjects had sufficient intakes of vitamin D and K based on AI in DRI 2005.

Multiple regression analyses for the determination of independent factor for circulating vitamin D, K concentrations.

In multiple regression analyses, vitamin D intake was a significant determinant of serum 25OH-D level, although the R^2 was low. Serum triglyceride level was the only significant predictor for plasma MK-7 concentration, and vitamin K intake and serum triglyceride concentrations significantly contributed to plasma PK level (Table 3).

Principal component analysis (PCA)

Since institutionalized elderly are generally malnourished, it is quite important to determine whether the low vitamin D - and K -status is independent of overall malnutrition or not. Then PCA was performed with the parameters included for analysis being serum albumin, triglyceride, cholesterol, 25OH-D, PTH levels and plasma PK, MK-7 concentrations. Four components were obtained and explained 82% of the variance. The first component was composite of high albumin, total cholesterol and 25OH-D, and second component consisted of high triglyceride, low

Table 3. Multiple regression analyses for the determination of independent factors for circulating vitamin D, K concentrations

	R^2	*p* value	Variable	β	*p* value
Serum 25OH-D	0.095	0.033	Vitamin D intake	0.309	0.033
Plasma PK	0.181	0.011	Vitamin K intake	0.290	0.042
			Triglyceride	0.380	0.009
Plasma MK-7	0.255	<0.001	Triglyceride	0.505	<0.001

Only significant predictors are shown. The abbreviations are β for β coefficient, and *p* for *p* value. Independent predictor for serum 25OH-D or plasma PK, MK-7 concentrations was analyzed by multivariate analysis with stepwise regression. Age, level of care needed and serum triglyceride and total cholesterol concentrations were included in all analyses. Vitamin D intake was additionally included in the analysis for plasma 25OH-D concentration. For plasma PK and MK-7, vitamin K intake was additionally included.

Table 4. Principal component analysis of nutrition indices

	Component 1	Component 2	Component 3	Component 4
Serum Albumin	0.880	0.004	0.047	0.059
Serum triglyceride	0.229	0.734	0.119	0.380
Serum total cholesterol	0.800	0.320	-0.046	-0.060
Serum 25OH-D	0.434	-0.457	-0.658	-0.033
Serum PTH	0.156	-0.273	0.877	-0.090
Plasma PK	-0.014	0.030	-0.071	0.986
Plasma MK-7	0.117	0.832	-0.238	-0.152

Factor loadings to four components after varimax rotation are shown. Loadings greater than 0.35 are shown in bold
Four components thus obtained were considered to represent the following nutritional status; component 1: overall nutritional status, component 2: vitamin K_2 status, component 3: vitamin D status, and component 4: vitamin K_1 status.

25OH-D, and high MK-7. The third component was composite of low 25OH-D and high PTH, and the fourth component was composed of high triglyceride and high PK. The interpretation of each component was made as follows; the first component representing overall nutritional status, the second component, vitamin K_2 status, the third component, vitamin D status, and the fourth component representing vitamin K_1 status (Table 4).

DISCUSSION

Nutritional status would be adequately assessed by both evaluating the subjects' food intake and measuring their circulating or urinary markers. This principle would hold true especially in the elderly, since they are at high risk for malabsorption or utilization defects of nutrients. Unfortunately in Japan, vitamin D and K status in the elderly has been studied either by evaluating their food intake, as in the annual National Nutrition Survey Japan (NNS-J) or by measuring circulating level of these vitamins,[21,30-33] but rarely by both.[12,34]

Institutionalized elderly have been our special concern, since they are much more susceptible to hypovitaminosis D and K deficiency than the healthy elderly. The NNS-J in 2006 showed that subjects over 70 years of age, including both genders, had the following daily nutrients intakes: energy 1761 kcal, calcium 551 mg, vitamin D 9.0 μg, vitamin K 273 μg,[35] which were higher than those of the subjects in the present study. Gastrointestinal absorption of nutrients in the present study subjects would be impaired also. These considerations led us to simultaneously evaluate both vitamin D and K intakes and its circulating levels in the present study.

Before the interpretation of our data, determination procedure for vitamin K deserves some discussion. There have been discrepancies on the plasma concentration of vitamin K in the previous literature, which is at least partly due to the different determination procedure employed. Recently we have developed a novel procedure for the determination of vitamin K analogs with high sensitivity and specificity, based on high-performance liquid chromatography-tandem mass-mass spectrometry with atmospheric pressure chemical ionization (LC-APCI-MS/MS).[26] With this procedure, plasma concentrations of PK and MK-7 were 0.73±0.70 ng/mL (median 0.58 ng/mL) and 0.53±0.37 ng/mL (median 0.45 ng/mL), respectively in the current study. In our recent study, plasma concentrations for PK and MK-7 were 1.29±1.09 ng/mL (median 0.94 ng/mL) and 4.21±6.81 ng/mL (median 2.14ng/mL), respectively in the healthy Japanese elderly over 70 years old using the same assay procedure.[21] In the same study, lowest concentration of plasma vitamin K level to avoid the elevation of serum ucOC concentration was 2.5 ng/ml for PK and 6.4 ng/ml for MK-7.[21] Since serum ucOC level is a sensitive indicator of skeletal vitamin K insufficiency, these figures can yield a rough estimate of circulating vitamin K levels needed by the skeleton.

The median intake of vitamin K in the current subjects was 168 μg, which was more than twice the AI in DRI 2005. The AI for vitamin K was not altered in DRI 2010. Dietary vitamin K intake has been identified as an important determinant of plasma phylloquinone concentration in previous studies.[36,37] In the present study, vitamin K intake was also significantly associated with plasma PK, but not with plasma MK-7. Since they were not supplied

with fermented soybean; natto, which contains exraordinary amount of MK-7,[38] phylloquinone from green vegetables is likely to be the major contributors to the total vitamin K intake in our subjects. Thus plasma PK alone correlated with total vitamin K intake, adjusted by serum triglyceride. These data strongly suggest that these subjects are vitamin K-deficient in spite of the fact that their dietary intake is far above the AI in according to DRI 2005, and increased vitamin K intake would be effective in improving plasma PK levels in institutionalized elderly in present study.

As in the case of vitamin K, average dietary intake of vitamin D was around 7 μg/day, which is approximately 140% of the AI in subjects in the present study. Nevertheless, the average serum 25OH-D concentration was only 11.1 ng/mL. Thus, most subjects in the present study had hypovitaminosis D in spite of apparently sufficient vitamin D intake.

Although the multiple regression analysis has identified vitamin D intake as the significant contributor to serum 25OH-D concentration, the R^2 value was low, which indicates that the current model could explain only a small portion of variation. Several factors could be responsible for the above results. First, because of walking disability and other physical dysfunction, the chance of sun exposure was minimal in most of the current study subjects, but it was not null. Thus, sun exposure may also partly explain the above results. Unfortunately, however, detailed information about sun exposure was unavailable. Furthermore, ADL itself has been reported to be related to serum 25OH-D levels,[39] on which detailed information is not available in the current study. Secondly, the intestinal absorption of vitamin D is likely to decrease due to factors such as compromised intestinal ability for nutrients absorption and limited fat intake.[40] Nevertheless, oral vitamin D intake seems to be of value in the institutionalized elderly for improving their vitamin D status. Cashman *et al.* reported dose-dependent increase in serum 25OH-D concentration after incremental supplementation with vitamin D_3 in free-living adults over 64 years of age.[41] Although AI for vitamin D slightly increased to 5.5 μg/day in recently issued DRI 2010, the elderly subjects are likely to require much more vitamin D intake to avoid hypovitaminosis D considering the various problems to interfere with absorption and utilization as discussed above. A second issue with regard to the above discussion; disturbed intestinal absorption and limited fat intake, will also apply to the discrepant intake and circulating level of vitamin K.

Although serum 25OH-D level was extremely low, average serum PTH level was within the reference range. Circulating 25OH-D concentrations showed significant negative correlation with serum PTH levels ($r= -0.293$, $p= 0.041$; data not shown), which suggests that the negative feedback regulation of PTH secretion by vitamin D is not impaired in the current population. Kuchuk *et al*. reported that the elevation of serum PTH concentration by vitamin D deficiency is moderate in its magnitude, and usually fell into the reference range.[42] Thus they stressed the importance of serum 25OH-D level, and argued that for bone health maintenance and physical performance in the elderly, serum 25OH-D concentration above 50-60 nmol/L (20-24 ng/mL) was required.

Although the institutionalized elderly are considered to be generally malnourished,[43-45] nutritional status appeared rather satisfactory in the present study subjects in face of hypovitaminosis D and K. Then we analyzed the relationship between the overall nutrition and circulating levels of vitamin D and K by PCA. The PCA have yielded four components representing: overall nutritional status, vitamin D status, vitamin K_2 status, and vitamin K_1 status respectively. Serum 25OH-D also exhibited some association with the first component, representing the overall nutritional status. One of the reasons for the above results would be that 25OH-D is bound to vitamin D-binding protein (DBP) and albumin during its transport in circulation.[46] Since these components are independent of each other by their definition, these results suggest that hypovitaminosis D and K in the institutionalized elderly do not merely reflect general malnutrition, and have their own role. Confounders are serious challenge in the clinical studies. In the intervention studies, randomization would eliminate the interference by the confounders. It would be less problematic in the case of cohort studies. Adjustment for confounders is quite difficult in the cross-sectional studies like the current one. Multivariate analyses such as PCA would be of help in eliminating the interference by confounders in this type of studies.

In conclusion, institutionalized elderly had high prevalence of hypovitaminosis D and K in spite of their dietary intake exceeding the AI in DRI 2005 in Japan, which suggests that the requirement for these vitamins would be higher in these subjects. Additionally, hypovitaminosis D and K were shown to be independent of general malnutrition by PCA, which would be a useful analytical procedure for eliminating the interference by confounders in cross sectional studies.

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AUTHOR DISCLOSURES

None of the authors have any conflicts of interest.

REFERENCES

1. Bilezikian JP, Silverberg SJ. The role of parathyroid hormone and vitamin D in the pathogenesis of osteoporosis. In: Marcus R, Feldman D, Kelsey J, editors. Osteoporosis 2nd ed. San Diego CA: Academic Press; 2001. pp.71-84.
2. Lips P. How to define normal values for serum concentrations of 25-hydroxyvitamin D? An overview. In: Feldman D, Pike JW, Glorieux FG, editors. Vitamin D 2nd ed. San Diego CA: Academic Press; 2005. pp. 1019-28.
3. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, et al. Effect of Vitamin D on falls: a meta-analysis. JAMA. 2004;291:1999-2006.
4. Kuroda T, Shiraki M, Tanaka S, Ohta H. Contributions of 25-hydroxyvitamin D, co-morbidities and bone mass to mortality in Japanese postmenopausal women. Bone. 2009; 44:168-72.

5. Eastell R, Lawrence Riggs B. Vitamin D and osteoporosis. In: Feldman D, Pike JW, Glorieux FG, editors. Vitamin D 2nd ed. San Diego CA: Academic Press; 2005. pp.1101-20.
6. Nakamura K, Nishiwaki T, Ueno K, Yamamoto M. Age-related decrease in serum 25-hydroxyvitamin D concentrations in the frail elderly: a longitudinal study. J Bone Miner Metab. 2007;25:232-6.
7. Lips P, van Ginkel FC, Jongen MJ, Rubertus F, van der Vijgh WJ, Netelenbos JC. Determinants of vitamin D status in patients with hip fracture and in elderly control subjects. Am J Clin Nutr. 1987;46:1005-10.
8. Lips P, Wiersinga A, van Ginkel FC, Jongen MJ, Netelenbos JC, Hackeng WH, et al. The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. J Clin Endocrinol Metab. 1988;67:644-50.
9. Suominem M, Laine T, Routasalo P, Pitkala KH, Rasanen L. Nutrient content of served food, nutrient intake and nutritional status of residents with dementia in a finnish nursing home. J Nutr Health Aging. 2004;8:234-8.
10. Przybelski R, Agrawal S, Krueger D, Engelke JA, Walbrun F, Binkley N. Rapid correction of low vitamin D status in nursing home residents. Osteoporos Int. 2008;19:1621-8.
11. Holick MF, Matsuoka LY, Wortsman J. Age, vitamin D, and solar ultraviolet. Lancet. 1989;2:1104-5.
12. Nashimoto M, Nakamura K, Matsuyama S, Hatakeyama M, Yamamoto M. Hypovitaminosis D and hyperparathyroidism in physically inactive elderly Japanese living in nursing homes: relationship with age, sunlight exposure and activities of daily living. Aging Clin Exp Res. 2002;14:5-12.
13. Vermeer C. Gamma-carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. Biochem J. 1990; 266:625-36.
14. Booth SL. Skeletal functions of vitamin K-dependent proteins: not just for clotting anymore. Nutr Rev. 1997;55:282-4.
15. Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr. 2000;71:1201-8.
16. Tsugawa N, Shiraki M, Suhara Y, Kamao M, Ozaki R, Tanaka K, et al. Low plasma phylloquinone concentration is associated with high incidence of vertebral fracture in Japanese women. J Bone Miner Metab. 2008;26:79-85.
17. Booth SL, Broe KE, Peterson JW, Cheng DM, Dawson-Hughes B, Gundberg CM, et al. Associations between vitamin K biochemical measures and bone mineral density in men and women. J Clin Endocrinol Metab. 2004;89:4904-9.
18. Seibel MJ, Robins SP, Bilezikian JP. Serum undercarboxylated osteocalcin and the risk of hip fracture. J Clin Endocrinol Metab. 1997;82:717-8.
19. Vergnaud P, Garnero P, Meunier PJ, Bréart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab. 1997; 82:719-24.
20. Sokoll LJ, Sadowski JA. Comparison of biochemical indexes for assessing vitamin K nutritional status in a healthy adult population. Am J Clin Nutr. 1996;63:566-73.
21. Tsugawa N, Shiraki M, Suhara Y, Kamao M, Tanaka K, Okano T. Vitamin K status of healthy Japanese women: age-related vitamin K requirement for gamma-carboxylation of osteocalcin. Am J Clin Nutr. 2006;83:380-6.
22. Sadowski JA, Hood SJ, Dallal GE, Garry PJ. Phylloquinone in plasma from elderly and young adults: factors influencing its concentration. Am J Clin Nutr. 1989;50:100-8.
23. Tse SL, Chan TY, Wu DM, Cheung AY, Kwok TC. Deficient dietary vitamin K intake among elderly nursing home residents in Hong Kong. Asia Pac J Clin Nutr. 2002;11:62-5.
24. Northstone K, Ness AR, Emmett PM, Rogers IS. Adjusting for energy intake in dietary pattern investigations using principal components analysis. Eur J Clin Nutr. 2008;62: 931-8.
25. Kesse-Guyot E, Bertrais S, Péneau S, Estaquio C, Dauchet L, Vergnaud AC, et al. Dietary patterns and their sociodemographic and behavioural correlates in French middle-aged adults from the SU.VI.MAX cohort. Eur J Clin Nutr. 2009;63:521-9.
26. Suhara Y, Kamao M, Tsugawa N, Okano T. Suhara Y, Kamao M, et al. Method for the determination of vitamin K homologues in human plasma using high-performance liquid chromatography-tandem mass spectrometry. Anal Chem. 2005;77:757-63.
27. Resources Council, Science and Technology Agency, Japan. Standard Tables of Food Composition in Japan, 5th ed. Tokyo: Printing Bureau, Ministry of Finance; 2000. 2009/11/4 [cited 2000/11/22]; Available from: http://www.mext.go.jp/english/news/2000/11/001150.htm#top. (in Japanese).
28. Ministry of Health, Labour, and Welfare, Japan. Dietary reference intakes for Japanese 2010. Tokyo: Daiichi-Shuppan; 2010. 2009/11/4 [cited 2009/5/29]; Available from: http://www-bm.mhlw.go.jp/shingi/2009/05/s0529-4.html. (in Japanese).
29. Ministry of Health, Labour, and Welfare, Japan. Dietary reference intakes for Japanese 2005. Tokyo: Daiichi-Shuppan; 2005. 2009/11/4 [cited 2004/11/22]; Available from: http://www.mhlw.go.jp/houdou/2004/11/h1122-2.html. (in Japanese).
30. Nakamura K, Nashimoto M, Yamamoto M. Summer/winter differences in the serum 25-hydroxyvitamin D3 and parathyroid hormone levels of Japanese women. Int J Biometeorol. 2000;44:186-9.
31. Nakamura K, Nashimoto M, Hori Y, Yamamoto M. Serum 25-hydroxyvitamin D concentrations and related dietary factors in peri- and postmenopausal Japanese women. Am J Clin Nutr. 2000;71:1161-5.
32. Nakamura K, Nashimoto M, Yamamoto M. Are the serum 25-hydroxyvitamin D concentrations in winter associated with forearm bone mineral density in healthy elderly Japanese women? Int J Vit Nutr Res. 2001;71:25-9.
33. Kaneki M, Hodges SJ, Hosoi T, Fujiwara S, Lyons A, Crean SJ, et al. Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. Nutrition. 2001;17:315-21.
34. Nakamura K, Tsugawa N, Saito T, Ishikawa M, Tsuchiya Y, Hyodo K, et al. Vitamin D status, bone mass, and bone metabolism in home-dwelling postmenopausal Japanese women: Yokogoshi Study. Bone.2008;42:271-7.
35. Ministry of Health, Labour, and Welfare, Japan. The National Nutrition Survey 2006. Tokyo: Daiichi-Shuppan; 2009. 2009/11/4 [cited 2008/04]; Available from: http://www.mhlw.go.jp/houdou/2008/04/h0430-2.html. (in Japanese).
36. McKeown NM, Jacques PF, Gundberg CM, Peterson JW, Tucker KL, Kiel DP, et al. Dietary and nondietary determinants of vitamin K biochemical measures in men and women. J Nutr. 2002;132:1329-34.
37. Shea MK, Benjamin EJ, Dupuis J, Massaro JM, Jacques PF, D'Agostino RB Sr, et al. Genetic and non-genetic correlates of vitamins K and D. Eur J Clin Nutr. 2009;63:458-64.
38. Ikeda H, Doi Y. A vitamin-K2-binding factor secreted from Bacillus subtilis. Eur J Biochem. 1990;28:219-24.
39. Nakamura K, Nishiwaki T, Ueno K, Yamamoto M. Serum 25-hydroxyvitamin D levels and activities of daily living in

noninstitutionalized elderly Japanese requiring care. J Bone Miner Metab.2005;23:488-94.
40. Kuwabara A, Tanaka K, Tsugawa N, Nakase H, Tsuji H, Shide K, et al. High prevalence of vitamin K and D deficiency and decreased BMD in inflammatory bowel disease. Osteoporos Int. 2009;20:935-42.
41. Cashman KD, Wallace JM, Horigan G, Hill TR, Barnes MS, Lucey AJ, et al. Estimation of the dietary requirement for vitamin D in free-living adults >=64 y of age. Am J Clin Nutr. 2009;89:1366-74.
42. Kuchuk NO, Pluijm SM, van Schoor NM, Looman CW, Smit JH, Lips P. Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. J Clin Endocrinol Metab.2009;94:1244-50.
43. Visvanathan R. Under-nutrition in older people: a serious and growing global problem!Visvanathan R. J Postgrad Med. 2003;49:352-60.
44. Kagansky N, Berner Y, Koren-Morag N, Perelman L, Knobler H, Levy S. Poor nutritional habits are predictors of poor outcome in very old hospitalized patients. Am J Clin Nutr. 2005;82:784-91.
45. Vellas B, Lauque S, Andrieu S, Nourhashemi F, Rolland Y, Baumgartner R, et al. Nutrition assessment in the elderly. Curr Opin Clin Nutr Metab Care. 2001;4:5-8.
46. Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E, Haddad JG. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. J Clin Endocrinol Metab. 1986;63:954-9.

Original Article

Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly

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居住機構中的老年人有高盛行率的維生素 D 及維生素 K 缺乏症且與整體的營養不良無相關

研究老年人的維生素 D 及維生素 K 缺乏症有許多方法學上的問題。首先，大多研究是藉由評估食物的攝取或是測量血中的濃度來進行的，但在日本很少同時利用這兩種方法。在本篇文章中，維生素 D 及維生素 K 的攝取以及老年人的血中濃度是同步測量的。第二個議題是維生素 D 及維生素 K 缺乏症是否與盛行於老年人的一般營養不良情形相關。我們試著藉由統計的主成份分析方法去分辨。評估 50 位機構中的老年人血中的 25-羥化維生素 D、副甲狀腺素、維生素 K_1、維生素 K_2 濃度，以及食物攝取。雖然平均維生素 D 攝取量(每天 7 克)超過日本所訂定的足夠攝取量(每天 5 克)，但平均血清中 25-羥化維生素 D 濃度(11.1 ng/mL)卻屬維生素 D 缺乏的範圍。維生素 K 攝取量的中位數為每天 168 克，這幾乎是維生素 K 的足夠攝取量的 2.5 倍。但是，血漿中維生素 K_1 及維生素 K_2 濃度是遠低於 70 歲以上健康的日本老人。應用主成份分析法，結果產生 4 個成份，分別代表整體營養狀況、維生素 K_2、維生素 D 及維生素 K_1 的營養狀況。既然每個成份都各自獨立，則這些老人的維生素 D 及維生素 K 缺乏不能用整體營養不良加以解釋。總之，在這些機構中的老年人具有高盛行率的維生素 D 及維生素 K 缺乏；爾後這類研究應該同時測量血中濃度及飲食攝取。主成份分析法，可排除橫斷性研究中其他干擾因子的作用，而得到有效的結果。

關鍵字：維生素 D 缺乏、維生素 K 缺乏、主成份分析、足夠攝取量、機構中的老年人

Original Article

Bone is more susceptible to vitamin K deficiency than liver in the institutionalized elderly

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In Japan, γ-carboxylation of blood coagulation factors is the basis for determining adequate intake (AI) for vitamin K in Dietary Reference Intakes (DRIs) issued in 2010. Recently, vitamin K is also known to be essential for preventing fracture. In this study, relative susceptibility of liver and bone to vitamin K deficiency was studied. Thirty-seven elderly institutionalized subjects were evaluated for vitamin K status by measuring serum PIVKA (protein induced by vitamin K absence) –II and ucOC (undercarboxylated osteocalcin) levels, as sensitive markers for hepatic and skeletal vitamin K deficiency, respectively. Serum PIVKA-II and ucOC levels, with their cut-off values in the parentheses, were 20.2±8.9 mAUmL (28 mAU/mL) and 4.7±3.0 ng/mL (4.5 ng/mL), respectively. Median vitamin K intake was approximately 200 μg/day, which is more than 3 times higher than the current Japanese AI. Vitamin K intake was significantly correlated with serum PIVKA-II and ucOC/OC levels, but not with serum ucOC level. Although serum ucOC level is generally a good indicator for vitamin K status, multiple regression analysis revealed that elevated bone turnover marker significantly contributed to serum ucOC level. All subjects had vitamin K intake exceeding AI for vitamin K. Nevertheless, serum PIVKA-II and ucOC concentrations exceeded the cut-off value in 14% and 43% of subjects, respectively. The present findings suggest that vitamin K intake greater than the current AI is required for the skeletal health in the institutionalized elderly.

Key Words: vitamin K, adequate intake, γ-carboxylation, ucOC, PIVKA-II

INTRODUCTION

Gamma-glutamyl carboxylase (GGCX) catalyzes the conversion of glutamyl (Glu) residue into γ-carboxyglutamyl (Gla) residue in certain proteins. The most fundamental role of vitamin K is the one as a cofactor of GGCX.[1] Although GGCX is present in various tissues, its role in the liver has received most attention until recently. In the liver, conversion of Glu residue to Gla residue takes place in four of the blood coagulation factors (II, VII, IX, and X), by which they acquire calcium-binding ability and are activated.[1] Recently, attention have been focused on the physiological roles of vitamin K-dependent proteins in extrahepatic tissues such as bone and blood vessel.[2,3] Osteocalcin is produced by osteoblasts, the most abundant non-collagenous protein in the bone matrix. Through γ-carboxylation, osteocalcin gains hydroxyapatite-binding ability, and regulates bone mineralization.[2] Recent evidences strongly suggest that skeletal vitamin K deficiency increases the risk of hip fracture.[4] Matrix Gla protein (MGP); another vitamin K-dependent protein, is an inhibitor of vascular calcification.[5-7]

In the current Japanese Dietary Reference Intakes (DRIs) issued in 2010, Adequate Intake (AI) for vitamin K in the adult is uniformly 75 μg/day for men and 65 μg/day for women. These values however, carries some problems when applied to the study population.[8] First, they are based on data from America or Europe. Since nutrients intake is greatly dependent on nationality or dietary patterns, vitamin K status in the Japanese must be studied. Second, they are from healthy young volunteers, not from the elderly who are likely to have nutrients malabsorption. This is especially the case with fat-soluble vitamins including vitamin K due to various factors such as decreased secretion of bile acids and pancreatic juice, and reduced dietary fat intake.[8] Finally, AI for vitamin K was determined as the dose sufficient to maintain normal blood coagulation with little mentioning to bone.[8] Serum levels of protein induced by vitamin K absence-II (PIVKA-II) and undercarboxylated osteocalcin (ucOC) are sensitive markers for vitamin K deficiency in the liver and bone, respectively. Vitamin K status in the liver and bone

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can be separately evaluated by measuring these markers. By employing such methodology, previous studies have shown that much higher doses of vitamin K are needed for the γ-carboxylation of osteocalcin than for that of blood coagulation factors.[9,10]

Thus it is possible that an elderly judged to be vitamin K sufficient based on the current AI has skeletal vitamin K deficiency and increased fracture risk. In this paper, we have measured serum PIVKA-II and ucOC levels, assessed vitamin K intake, and studied the prevalence of vitamin K deficiency in the liver and bone in the institutionalized elderly.

MATERIALS AND METHODS

Subjects

The study subjects were 37 institutionalized elderly (male 8, female 29) in a nursing home, Kayu-Shirakawa. Exclusion criteria were routine medication that has potential interference with bone metabolism and vitamin K status such as warfarin. None had history of hepatic diseases. Detailed information about this study was given and written consent was obtained from the subject or the proxy. The study protocol was approved by the ethical committee in Kyoto Women's University.

Laboratory data

Blood was obtained after overnight fasting. After centrifugation, serum was kept frozen at –30°C until analysis. Serum PIVKA-II and ucOC levels were measured by electro chemiluminescence immunoassay (ECLIA) (Sanko Junyaku, Co, Ltd, Tokyo, Japan) as the markers of hepatic and skeletal vitamin K deficiency, respectively. Serum intact osteocalcin (intact OC) was measured by enzyme immunoassay (EIA) (Mitsubishi Yuka, Tokyo, Japan). The ucOC/OC was calculated as the ratio of ucOC to intact OC. Serum levels of tartrate-resistant acid phosphatase-5b (TRACP-5b) and bone specific alkaline phosphatase (BAP) were measured by EIA (DS Pharma Biomedical, Osaka, Japan) and chemiluminescence enzyme immunoassay (CLEIA) (Beckman Coulter Inc, Tokyo, Japan), respectively. TRACP-5b and BAP are markers of bone resorption and bone formation, respectively. The reference range of serum TRACP-5b was 170-590 mU/dL in male and 120-420 mU/dL in female, and that of serum BAP was 3.7-20.9 μg/L in male and 3.8-22.6 μg/L in female.

Nutrition intake study

Nutrient intake was assessed by food record method. The intake of vitamin K was calculated by multiplying the amount of vitamin K supplied from the institution with the average percentage intake. Based on these records, their intake of vitamin K was calculated using the software (Healthy Maker Pro 501, Mushroom Software Corp, Okayama, Japan). Vitamin K intake/kg body weight was also calculated, since 1 μg/kg of vitamin K is considered to be sufficient for maintaining normal coagulation in the adult according to the Japanese DRI 2010.[8]

Statistical analyses

Statistical analyses were performed using the SPSS 17.0 J for Windows (SPSS. Japan Inc, Tokyo, Japan). Association between variables was analyzed by Pearson's or Spearman rank correlation coefficient. Multiple regression analyses with stepwise method were performed to determine independent determinants for serum ucOC and ucOC/OC. Chi-square test was employed for categorical data.

RESULTS

Background profiles of the study subjects

The background profiles and biochemical data are shown in Table 1. Care level is a 5-grade score which is commonly used in the long-term care insurance in Japan with higher number indicating more intensive care needed. It was higher than grade 3 in 78% of subjects, indicating that they had low physical activity level. For example, most of the present subjects required wheelchair for transportation. In 27% of subjects, serum albumin level was lower than 3.5 g/dL, which is a generally accepted cut-off for malnutrition. Overall, nutritional parameters including the biochemical indicators and body mass index (BMI) remained within the reference range for most of the subjects. Thus, despite the elderly population and high level of care needed, the subjects' nutritional status was considered to be generally preserved. Although average serum TRACP-5b and BAP levels were within the reference range as a whole, 20% and 32% of subjects had serum BAP and TRACP-5b level above upper reference range, respectively. Serum PIVKA-II and ucOC levels were 20.2±8.9 mAU/mL and 4.7±3.0 ng/mL, respectively. All subjects were on orally consumed their meals. Although energy intakes were lower than estimated energy requirement (EER) of DRI in all men and 93% of women, the intake of macronutrients such as protein, fat and carbohydrates appeared appropriate for their age and sex. Average vitamin K intake was 194±51 (median; 197)

Table 1. Baseline data of the study subjects

	(M/F; 8/29, n=37)
Age (y)	85.1±8.2（87.0）
Care level	Median; 3 (min-max; 1-5)
Body weight (kg)	45.9±6.1（46.1）
Height (cm)	149.3±9.7（145.3）
BMI (kg/m^2)	20.6±2.5（20.0）
Serum Albumin (g/dL)	3.7±0.3（3.8）
Serum triglyceride (mg/dL)	119±41（118）
Serum total cholesterol (mg/dL)	198±49（191）
eGFR (ml/min./1.73m^2)	65.4±15.8（63.3）
Serum BAP (μg/L)	18.4±9.6（17.6）
Serum TRACP-5b (mU/dL)	365.2±124.9（372.0）
Serum ucOC (ng/mL)	4.7±3.0 (3.8)
Serum total OC (ng/mL)	6.1±3.1 (5.4)
ucOC / intact OC	0.81±0.36 (0.80)
Serum PIVKA-II (mAU/mL)	20.2±8.9 (18.0)
Energy intake (kcal)	1346±129 (1401)
Protein intake (g)	53.2±5.2 (55.4)
Fat intake (g)	35.6±3.6 (36.9)
Carbohydrates intake (g)	193.8±18.7 (199.4)
Vitamin K intake (μg/day)	194±51 (197)
Vitamin K intake/BW (μg/BW kg/day)	3.5±1.1 (3.4)

Data are expressed as mean±SD with the values in parentheses showing the median.

μg/day in the study population, 166±50 (median; 159) μg/day in males and 202±49 (median; 224) μg/day in females. It was approximately 220% and 310% of the AI in DRI in male and female subjects, respectively. All subjects had vitamin K intake exceeding AI. In addition, the vitamin K intake/kg body weight was 3.5±1.1 μg/day in the present study subjects, far exceeding 1μg/kg.

Correlations among vitamin K intake and serum PIVKA-II, OCs

Table 2 shows that vitamin K intake was significantly correlated with serum PIVKA-II and ucOC/OC levels, but not with serum ucOC concentrations. (Table 2)

Correlations among serum OCs and bone turnover markers

Serum TRACP-5b and BAP levels were significantly correlated with serum ucOC concentration, but not with ucOC/OC ratio. (Table 3)

Multiple regression analyses for serum OCs levels

Multiple regression analyses revealed that serum TRACP-5b level was a significant determinant of serum ucOC concentration. Vitamin K intake was a significant predictor for ucOC/OC. (Table 4)

Relative susceptibility of liver and bone to vitamin K deficiency

Serum PIVKA-II level exceeded the cut-off level (28 mAU/mL) in only 14% of the subjects, whereas serum ucOC concentration was above the cut-off value (4.5 ng/mL) in 43% of subjects, which was significantly different by chi-square test (p<0.001). (Table 5)

DISCUSSION

Vitamin status could be evaluated by several ways such as measuring its blood concentration or measuring the markers representing the vitamin status. Recently, we have reported that the prevalence of vitamin D- and K-deficiency is quite high in the institutionalized elderly by measuring plasma levels of 25 hydroxy-vitamin D concentration which is the best indicator of vitamin D status, and plasma vitamin K concentration.[11] Plasma vitamin K concentrations, however, only reflect the vitamin K status as a whole, and do not provide us with information regarding the vitamin K status in various tissues individually. Thus, in this study, we have evaluated the subjects' vitamin K status by measuring their serum levels of PIVKA- II and ucOC rather than their plasma vitamin K levels.

First, we have studied the association between serum levels of PIVKA-II and ucOC, and vitamin K intake. Vitamin K intake was significantly correlated with PIVKA-II and ucOC/OC, but not with ucOC. Similar findings were also reported by Booth *et al* that circulating levels of PIVKA-II and ucOC/OC ratio reflected dietary vitamin K intake, whereas serum ucOC levels did not.[9] Two mechanisms were considered to be responsible for these find-

Table 2. The correlation between vitamin K intake and serum levels of PIVKA-II and ucOC

	ucOC		ucOC/OC		PIVKA-II	
	r	*p*-value	r	*p*-value	r	*p*-value
Vitamin K intake	0.092	0.588	-0.416	0.010	-0.362	0.028

Correlations of vitamin K intake with markers for vitamin K deficiency were analyzed by Spearman rank correlation.

Table 3. The correlation of serum ucOC and uc/OC ration and bone turnover markers

	ucOC		ucOC/OC	
	r	*p*-value	r	*p*-value
Serum TRACP-5b	0.425	0.009	0.014	0.935
Serum BAP	0.517	0.001	0.243	0.147

Correlations of serum OCs with bone turnover markers were analyzed by Spearman rank correlation.

Table 4. Multiple regression analyses for serum ucOC level and ucOC/OC ratio

Dependent variable	R^2	Independent variable	β	*p*-value
ucOC	0.206**	Serum TRACP-5b	0.454	0.005
ucOC/OC	0.134*	Vitamin K	-0.366	0.026

The abbreviations are β for β coefficient. Independent predictor(s) for serum OCs levels were analyzed by multiple regression analyses with stepwise method. Sex, serum TRACP-5b, and vitamin K intake (μg) were included in all analyses.
*; p< 0.05, **; p< 0.01

Table 5. Number of subjects with vitamin K sufficiency and deficiency in the liver and bone

	Vitamin K sufficiency	Vitamin K deficiency
In the bone (serum ucOC concentration)	21 (57%)	16 (43%)
In the liver (serum PIVKA-II concentration)	32 (86%)	5 (14%)

Values represent number of subjects, with percentage of subjects in the parentheses. Vitamin K status in the bone and that in the liver were significantly different by chi-square test (p<0.001).

ings. The first is the different bioavailability of phylloquinone (PK; vitamin K_1) and menaquinones (MKs; vitamin K_2). In the present study, PK was the major form of vitamin K taken as in America or Europe,[12,13] since the subjects had no intake of natto which contains large amount of MK-7 during the study.[14] Recent studies have shown that PK can be utilized for γ-carboxylation in the liver, but can only be utilized in extrahepatic tissues after conversion into MK-4.[15,16]

Second issue is the association of serum ucOC level with bone turnover. Serum levels of BAP and TRACP-5b reflect osteoblastic bone formation and osteoclastic bone resorption, respectively, and are elevated in the high turnover state. Since osteocalcin is produced in osteoblasts,[17] it is conceivable that serum concentration of osteocalcin as well as its subfraction, ucOC level is increased with high turnover. Thus, it is currently under debate whether ucOC alone is satisfactory or measurement of ucOC as well as ucOC/OC is a better indicator of vitamin K status. In the present study, vitamin K intake was a significant predictor for ucOC/OC, but not with ucOC. Therefore, there is a possibility that ucOC/OC is a better index for vitamin K status than serum ucOC concentration. Unfortunately, however, there is no cut-off value published regarding ucOC/OC ratio, while the clinical usefulness of serum ucOC measurement is increasingly acknowledged. Thus, analysis using ucOC/OC could not be done as serum ucOC level in Table 5.

The cut-off value of 4.5 ng/mL for serum ucOC was validated by Shiraki by simultaneously evaluating the subjects' dietary intake of vitamin K, blood levels of vitamin K and ucOC.[18] They also reported that serum ucOC concentration exceeding 5.5 ng/mL was associated with increased risk of fracture. The clinical usefulness of ucOC measurement was previously reported, although with different assay procedure of hydroxy-appatite binding assay. In the European epidemiological study, Vergnaud *et al* reported that subjects in the lowest quartile of femoral neck bone mineral density (BMD) and those in the highest quartile of ucOC had increased hip fracture risk with an odds ratio of 2.4 and 1.9, respectively. These two risk factors were independent of each other, and those with both conditions had a even higher odds ratio of 5.5.[19] Thus, serum ucOC concentration is shown to be a good indicator of skeletal vitamin K deficiency, and a predictor of fracture risk.

In the current study subjects with vitamin K intake far exceeding AI, serum concentration of PIVKA-II and ucOC were within the reference range in 86% and 57% of the subjects respectively, which was significantly different. Thus, their vitamin K intake is sufficient for γ-carboxylation in the liver, but not in the bone, and bone is much more susceptible to vitamin K deficiency than liver. Such difference is likely to arise from the anatomical basis that vitamin K absorbed from the intestine is first transported to liver and preferentially used there, then utilized in extrahepatic organs.[9,10]

Booth *et al* in their depletion-repletion studies, reported that the γ-carboxylation of prothrombin was restored at 200 μg/day of PK, whereas that of osteocalcin was not even at 450 μg/day of PK.[9] Schurgers *et al* also reported that undercarboxylated prothrombin concentration was significantly decreased at supplementary intake of 100 μg/day of PK, whereas ucOC level did not decrease below 300 μg/day of PK.[10] Furthermore, Binkley *et al* reported that supplementation with 1,000 μg/day of vitamin K was optimal for the maximal γ-carboxylation of osteocalcin.[20] These results suggest that at least 300-500 μg g/day of vitamin K intake is required for the sufficient γ-carboxylation in the bone. Our results in the Japanese elderly are compatible with these results from Caucasians, and have additionally provided data on the prevalence of hepatic and skeletal vitamin K deficiency.

We believe that this paper is of importance in considering the AI for vitamin K. The current DRI states that the AI for vitamin K was determined based on its requirement for the γ-carboxylation of blood coagulation factors. The present findings suggest that vitamin K intake greater than the current AI is required for the skeletal health in the institutionalized elderly. Further studies with lager number of subjects and intervention studies are necessary to define the amount of vitamin K necessary for the elderly.

AUTHOR DISCLOSURES

None of the authors have any conflicts of interest.

REFERENCES

1. Vermeer C. γ-Carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. Biochem J. 1990;266: 625-36.
2. Booth SL. Skeletal functions of vitamin K-dependent proteins: not just for clotting anymore. Nutr Rev. 1997;55:282-4.
3. Vermeer C, Shearer MJ, Zittermann A, Bolton-Smith C, Szulc P, Hodges S, Walter P, Rambeck W, Stöcklin E, Weber P. Beyond deficiency: potential benefits of increased intakes of vitamin K for bone and vascular health. Eur J Nutr. 2004;43:325-35.
4. Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr. 2000;71:1201-8.
5. Danziger J. Vitamin K-deoendent proteins, warfarin, and vascular calcification. Clin J Am Soc Nephrol. 2008;3:1504-10.
6. Proudfot D, ShanahanCM. Molecular mechanisms mediating vascular calcification: role of matrix Gla protein. Nephrology. 2006;11:455-61.
7. Shanahan CM, Proudfoot D, Farzaneh-Far A, Weissberg PL. The role of Gla proteins in vascular calcification. Crit Rev Eukaryot Gene Expr. 1998;8: 357-75.
8. Ministry of Health, Labour, and Welfare, Japan. Dietary reference intakes for Japanese 2010. Tokyo: Daiichi-Shuppan; 2010 (in Japanese).
9. Booth SL, Martini L, Peterson JW, Saltzman E, Dallal GE, Wood RJ. Dietary phylloquinone depletion and repletion in older women. J Nutr. 2003;133:2565-9.
10. Schurgers LJ, Shearer MJ, Hamulyák K, Stöcklin E, Vermeer C. Effect of vitamin K intake on the stability of oral anticoagulant treatment: dose-response relationships in healthy subjects. Blood. 2004;104:2682-9.
11. Kuwabara A, Himeno M, Tsugawa N, Kamao M, Fujii M, Kawai N et al. Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly. Asia Pac J Clin Nutr. 2010;19:49-56.

12. Kamao M, Suhara Y, Tsugawa N, Uwano M, Yamaguchi N, Uenishi K, Ishida H, Sasaki S, Okano T. Vitamin K content of foods and dietary vitamin K intake in Japanese young women. J Nutr Sci Vitaminol. 2007;53:464-70.
13. Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. J Nutr. 1998;128:785-8.
14. Kaneki M, Hodges SJ, Hosoi T, Fujiwara S, Lyons A, Crean SJ et al. Japanese fermented soybean food as the major determination of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. Nutrition. 2001;17:315-21.
15. Okano T, Shimomura Y, Yamane M, Suhara Y, Kamao M, Sugiura M, Nakagawa K. Conversion of phylloquinone (Vitamin K1) into menaquinone-4 (Vitamin K2) in mice: two possible routes for menaquinone-4 accumulation in cerebra of mice. J Biol Chem. 2008;283:11270-9.
16. Thijssen HH, Vervoort LM, Schurgers LJ, Shearer MJ. Menadione is a metabolite of oral vitamin K. Br J Nutr. 2006; 95:260-6.
17. Price PA, Otsuka AA, Poser JW, Kristaponis J, Raman N. Characterization of a gamma-carboxyglutamic acid-containing protein from bone. Proc Natl Acad Sci U S A. 1976;73: 1447-51.
18. Shiraki M, Aoki C, Yamazaki N, Ito Y, Tsugawa N, Suhara Y, Okano T. Clinical assessment of undercarboxylated osteocalcin measurement in serum using an electrochemiluminescence immunoassay: Establishments of cut-off values to determine vitamin K insufficiency in bone and to predict fracture leading to clinical use of vitamin K_2. Iyaku to yakugaku. 2007;57:537-46. (in Japanese)
19. Vergnaud P, Garnero P, Meunier PJ, Bréart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab. 1997;82: 719-24.
20. Binkley NC, Krueger DC, Kawahara TN, Engelke JA, Chappell RJ, Suttie JW. A high phylloquinone intake is required to achieve maximal osteocalcin γ-carboxylation. Am J Clin Nutr. 2002;76:1055-60.

Original Article

Bone is more susceptible to vitamin K deficiency than liver in the institutionalized elderly

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居住機構老人骨骼比肝臟易受維生素 K 缺乏影響

日本 2010 年發佈的膳食營養素參考攝取量(DRI)中，維生素 K 的足夠攝取量是根據凝血因子的 γ-羧化作用而訂定的。近來，維生素 K 也被視為預防骨折不可或缺的角色。本研究在於比較肝和骨骼對維生素 K 缺乏的敏感性。評估 37 位居住機構的老人之維生素 K 狀況─測量血清 PIVKA–II (因維生素 K 缺乏所產生的蛋白質)和 ucOC (未羧化的骨鈣素)濃度，兩者分別為肝和骨骼在維生素 K 缺乏時的敏感指標。受試者血清 PIVKA-II 和 ucOC 濃度分別為 20.2±8.9 mAU/mL (臨界值 28 mAU/mL)和 4.7±3.0 ng/mL (臨界值 4.5 ng/mL)。維生素 K 攝取量中位數約為 200 μg/day，超過了日本目前所建議的足夠攝取量 3 倍。維生素 K 攝取量與血清 PIVKA-II 和 ucOC/OC 濃度顯著相關，但與血清 ucOC 濃度無相關。雖然血清 ucOC 濃度是體內維生素 K 狀況很好的指標，但複迴歸分析顯示骨骼轉換標記增加，也會影響血清 ucOC 濃度。所有的受試者維生素 K 攝取量皆超過足夠攝取量。然而，分別有 14%和 43%受試者的血清 PIVKA-II 和 ucOC 濃度超過臨界值。本研究結果建議，對於住在機構的老人，為維持骨骼健康，維生素 K 攝取量應超過目前建議的足夠攝取量。

關鍵字：維生素 K、足夠攝取量、γ-羧化作用、未羧化骨鈣素、PIVKA-II

ビタミンK

Vitamin K treatment for osteoporosis

特集

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すべての医師のための骨粗鬆症診療ガイド2010
Key words ビタミンK　ucOC(undercarboxylated osteocalcin)　menaquinone-4

ビタミンKの同族体(図1)

ビタミンKは，ビタミンK_1(phylloquinone；PK)とビタミンK_2(menaquinones；MK)に分けられる．いずれもナフトキノン骨格に側鎖が付いた構造を持ち，骨格部分は両者で共通だが，側鎖は両者で大きく異なる．PKは1種類のみであり，緑色野菜に多く含まれる．一方，MKは動物性食品に多く含まれ，側鎖の異なる同族体が多数存在し，側鎖の長さによってMK-nのように呼ばれる．腸内細菌は側鎖の長いビタミンK_2を合成し，とくにMK-7は納豆に豊富に含まれる．骨粗鬆症治療薬として臨床で用いられるグラケー®(エーザイ)は1錠にMK-4を15mg含み，1日量45mgである．

最近，PKとMKの相違点が明らかになってきており，岡野らによって，他のビタミンK同族体は，生体内でMK-4に変換されて作用することが示されている[1]．またMK(とくにMK-7)は，生体内での半減期が長いため，PKより効力が大きい[2]．

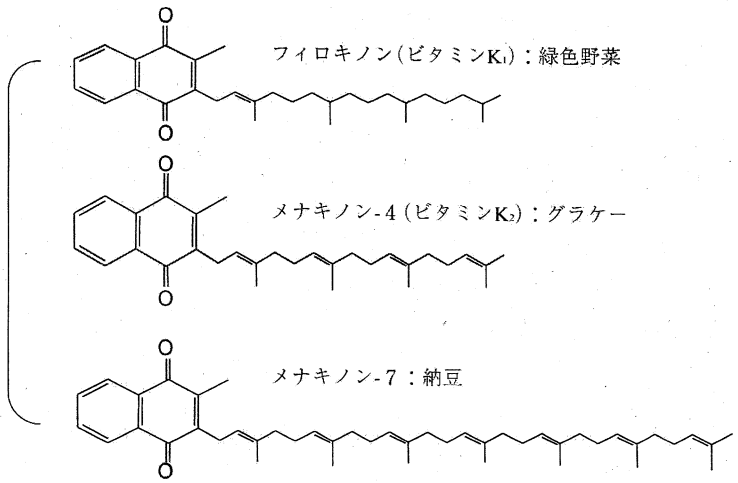

図1　ビタミンK

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0371-1900/10/¥50/頁/JCOPY

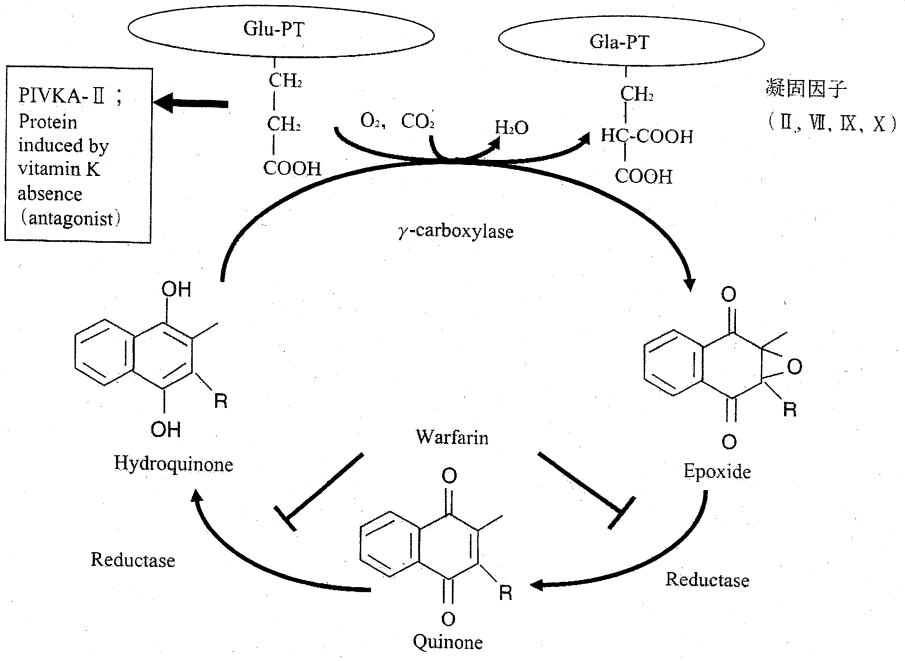

図2 肝臓におけるビタミンのK作用

ビタミンKの作用(図2)

ビタミンKの最も基本的作用は，酵素γ-carboxylase(GGCX)の補酵素として働くことである．GGCXは肝臓において，血液凝固因子のうちⅡ(プロトロンビン；図中ではPT)・Ⅶ・Ⅸ・Ⅹ因子のグルタミン酸残基(Glu)に，新たにカルボキシル基を導入してγ-カルボキシルグルタミン酸(Gla)残基に変える．これらの因子はGla型になってカルシウムイオン結合能を獲得し，凝固因子としての作用を発揮しうる．ビタミンKは補酵素として作用すると酸化されるが，ビタミンKサイクルによって還元され，何度も再利用される．抗凝固剤のワルファリンは，このサイクルの阻害が作用機構である．

近年，肝臓以外での重要性を示す報告が相次いでいる．GGCXによってGla化されるたんぱく質は，凝固因子以外にも多数存在する．オステオカルシンは，骨基質たんぱく質としてはコラーゲンに次いで多いものであるが，ビタミンKを補酵素として，GGCXによってGla化される．また，matrix Gla protein(MGP)は血管・骨に存在するGlaたんぱく質であり，血管の石灰化抑制因子である．さらにMKに関しては，核内受容体SXRを介する作用も報告されている．

ビタミンKと骨折の関連(疫学研究)

疫学調査から，骨折予防におけるビタミンKの意義を示す論文がいくつか報告されている．ビタミンK摂取量によって対象者を4群に分けて分析したところ，ビタミンK摂取量の最も高かった群の骨折リスクは，最も低かった群のリスクの半分以下であった[3]．また，骨密度低下によって大腿骨頸部骨折のリスクは2.4倍，骨におけるビタミンK作用不足の指標である血清ucOC(undercarboxylated osteocalcin；Gla化されていないオステオカルシン)濃度高値によって1.9倍に増加し，骨密度低下とucOC高値の両方を有する例では，骨折のリスクは5.5倍にも上昇することが報

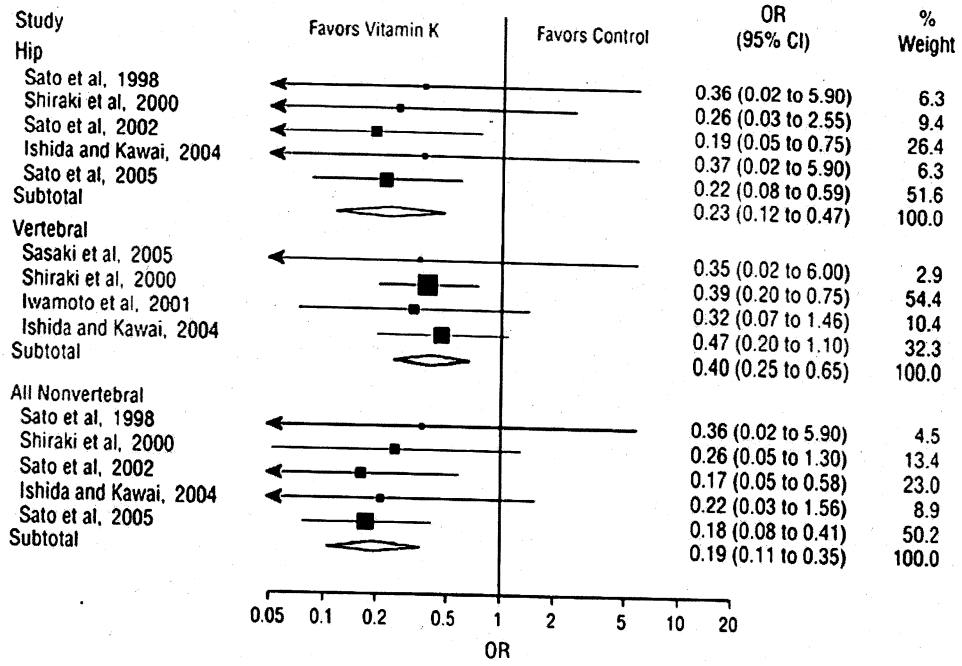

図3 ビタミンK治療による骨折抑制(メタアナリシス)
(Cockayne S, et al：Vitamin K and the Prevention of Fractures. Systematic Review and Meta-analysis of Randomized Control Trials. Arch Intern Med 166：1256-1261, 2006)

告され，このことは，ucOC高値すなわち骨におけるビタミンKの作用不足は，骨密度とは独立した骨折の危険因子であることを示す[4]．

ビタミンKによる骨折の抑制(介入試験)

白木は，241例の骨粗鬆症患者をランダムに，ビタミンK_2 45mg/day投与と対照群に割り付けて2年間フォローしたところ，ビタミンK_2投与群においては，ucOCが低下し，骨密度低下が防止され，さらに新規骨折発生が有意に減少した[5]．ビタミンK介入による骨折抑制に関しては，すでにメタアナリシスが発表されており，著明な骨折抑制効果が報告されている[6](**図3**)．これは主に，45mgのビタミンK_2に関するものであるが，最近5mgのビタミンK_1による骨折発生抑制効果も報告されている[7]．

最近わが国において，ビタミンK_2による骨折抑制効果を検証する大規模調査，OF(Osteoporosis Fracture) Studyの結果が発表された[8]．対象例全体では，椎体骨折の新規発生は，ビタミンK_2投与群で抑制されたとは言えないが，椎体骨折数5個以上を有する群においては有意の抑制が見られ，身長低下の抑制は，75歳以上・閉経後30年以上・椎体骨折数5個以上の群で見られた．

ビタミンKによる骨折抑制作用の指標

骨強度は，骨密度と骨質の両者によって規定されるが，ビタミンKは，骨密度を顕著に増加させる薬剤ではない．臨床的に骨密度を指標として薬効判定を行うことは困難であり，それ以外の評価指標が必要である．骨質の面から考えざるを得ないが，現時点では，非侵襲的に骨質を簡便に評価する機器はない．Knapenらは，DXA法を用いて骨密度を測定するとともに骨強度の指標を算

出し，ビタミンKは，骨強度指標を改善すると報告している[9]．

しかしこの方法も，実地臨床において患者の評価に用いるのは，なお困難であり，血液・尿検査による評価ができれば，より現実的である．血中ビタミンK濃度測定を実地に用いるのは困難であり，臨床的にはビタミンK不足によって血液中濃度が変化する物質を測定して，これを代替マーカーとするのが現実的であり，それがucOCである．

最近，血清ucOCの簡便な測定キットが発売・保険収載され，実際に臨床現場で用いられるようになった(三光純薬，ピコルミucOC)．白木らは，このキットを用いた場合のカットオフ値を4.5 ng/mLとし，5.5ng/mL以上では骨折のリスクが増大したと報告している[10]．津川らは，日本人の健常女性396名を対象とした報告において，血清ucOC濃度は血漿ビタミンK_1およびMK-7濃度と有意な関係を認めている[11]．

ごく最近白木らは，ビタミンK投与によって，骨形成マーカーが増加し，代謝回転を促進することを報告しており，ビタミンKの効果判定において，新たな指標となる可能性がある[12]．

ビタミンKの効果の不均一性

ビスフォスフォネートやSERMに比べると，ビタミンKの有効性に関しては，報告ごとのばらつきが大きい．有効性を示したメタアナリシス[6]に対しても異論がある．その原因は，現時点ではまだ明らかではないが，いくつかの要因が考えられる．

GGCXには遺伝子多型があり，それによって，酵素活性が異なることが報告されている[13]

またビタミンである以上，欠乏者に対しては，より大きな効果を発揮することは考えられる．佐藤らは，アルツハイマー病女性患者に対し，ビタミンK_2・D_2・カルシウム補給を行ったところ，非椎体骨折の発生が著しく抑制されたと報告している[14]．上記OF Studyの結果[8]も，複数骨折を有する重症例にビタミンK_2が有効というよりは，これらの例は高齢の，ビタミン欠乏の頻度が高いと考えられる例であり，ビタミンK欠乏例に対しては，骨折予防効果が明らかに認められると解釈されるのかもしれない(著者私見)．

骨粗鬆症治療におけるビタミンKの位置づけ

「骨粗鬆症の予防と治療ガイドライン(2006年版)」において，ビタミンKは，骨密度：わずかではあるが増加効果がある(グレードB)，椎体骨折：椎体骨折を防止するとの報告がある(グレードB)，非椎体骨折：非椎体骨折を防止するとの報告がある(グレードB)，(総合評価：グレードB)となっており[15]，さらに「骨密度の増加を介さない骨折予防効果が期待される．疫学的データもビタミンK_2による骨折予防効果を支持するものの，さらに骨折やQOLをエンドポイントとした前向き研究が望まれる」との付記がある．

ビタミンKは，ビスフォスフォネートなどの骨吸収抑制剤とはまったく異なる機構で作用する薬剤であり，単剤での投与だけではなく，併用による効果も期待される．現在，A-TOP研究会によるJOINT-03プロジェクトにより，リセドロネートとの併用効果に関する研究が進行中である．

また，ビタミンK治療の医療経済評価についての大部な論文も最近発表されているが，現時点でのエビデンスでは，判断困難のようである[16]．

文　献

1) Okano T, Shimomura Y, Yamane M, et al：Conversion of phylloquinone (Vitamin K_1) into menaquinone-4 (Vitamin K_2) in mice：two possible routes for menaquinone-4 accumulation in cerebra of mice. J Biol Chem 283：11270-11279, 2008.

2) Schurgers LJ, Teunissen KJ, Hamulyák K, et al：Vitamin K－containing dietary supplements：comparison of synthetic vitamin K_1 and natto-derived menaquinone-7. Blood 109：3279-3283, 2007.
3) Booth SL, Tucker KL, Chen H, et al：Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr 71：1201-1208, 2000.
4) Vergnaud P, Garnero P, Meunier PJ, et al：Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women：the EPIDOS Study. J Clin Endocrinol Metab 82：719-724, 1997.
5) Shiraki M, Shiraki Y, Aoki C, et al：Vitamin K_2 (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. J Bone Miner Res 15：515-521, 2000.
6) Cockayne S, Adamson J, Lanham-New S, et al：Vitamin K and the prevention of fractures：systematic review and meta-analysis of randomized controlled trials. Arch Intern Med 166：1256-1261, 2006.
7) Cheung AM, Tile L, Lee Y, et al：Vitamin K supplementation in postmenopausal women with osteopenia (ECKO trial)：a randomized controlled trial. PLoS Med 5(10)：e196, 2008.
8) Inoue T, Fujita T, Kishimoto H, et al：Randomized controlled study on the prevention of osteoporotic fractures (OF study)：a phase IV clinical study of 15-mg menatetrenone capsules. J Bone Miner Metab 27：66-75, 2009.
9) Knapen MHJ, Schurgers LJ, Vermeer C：Vitamin K_2 supplementation improves hip bone geometry and bone strength indices in postmenopausal women. Osteoporos Int 18：963-972, 2007.
10) 白木正孝，青木長寿，山崎典美ほか：電気化学発光免疫法による血清中低カルボキシル化オステオカルシン(ucOC)測定キットの臨床的有用性の検討－カットオフ値の設定と骨粗鬆症患者におけるビタミンK_2剤選択時の有用性の検討－．医学と薬学 57：537-546，2007.
11) Tsugawa N, Shiraki M, Suhara Y, et al：Vitamin K status of healthy Japanese women：age-related vitamin K requirement for gamma-carboxylation of osteocalcin. Am J Clin Nutr 83：380-386, 2006.
12) Shiraki M, Itabashi A：Short-term menatetrenone therapy increases gamma-carboxylation of osteocalcin with a moderate increase of bone turnover in postmenopausal osteoporosis：a randomized prospective study. J Bone Miner Metab 27：333-340, 2009.
13) Kinoshita H, Nakagawa K, Narusawa K, et al：A functional single nucleotide polymorphism in the vitamin-K-dependent gamma-glutamyl carboxylase gene (Arg325Gln) is associated with bone mineral density in elderly Japanese women. Bone 40：451-456, 2007.
14) Sato Y, et al：Menatetrenone and vitamin D_2 with calcium supplements prevent nonvertebral fracture in elderly women with Alzheimer's disease. Bone 36：61-68, 2005.
15) 骨粗鬆症の予防と治療ガイドライン作成委員会：骨粗鬆症の予防と治療ガイドライン(2006年版)ライフサイエンス出版 2006 (医療情報サービス Minds から読むことができる http://minds.jcqhc.or.jp/stc/0046/1/0046_G0000129_GL.html)
16) Stevenson M, Lloyd-Jones M, Papaioannou D：Vitamin K to prevent fractures in older women：systematic review and economic evaluation. Health Technol Assess 13(45)：1-134, 2009.

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Original Article

Tissue accumulation and urinary excretion of chromium in rats fed diets containing graded levels of chromium chloride or chromium picolinate

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ABSTRACT — To attempt a risk assessment of the excess intake of trivalent chromium (Cr), tissue Cr accumulation and urinary Cr excretion were examined in weanling rats fed experimental diets containing graded levels of Cr chloride ($CrCl_3$) or Cr picolinate (CrPic). Thirty-six male weanling 4-weeks-old Wistar rats were divided into six groups and fed a casein-based semi-purified diet (Cr content: < 0.02 μg/g) supplemented with 1, 10, or 100 μg Cr/g as $CrCl_3$ or CrPic for 28 days. Among the experimental groups, no significant difference was observed in body weight; however, supplementation of 100 μg Cr/g to the diets caused a significant low liver weight irrespective of the chemical species of Cr. Activities of serum aspartate aminotransferase and alanine aminotransferase were significantly elevated in rats given CrPic at 100 μg Cr/g. In the liver, kidney and femur, Cr accumulation increased with elevation of the dietary Cr level. No influence of the difference in the chemical species of supplemented Cr was observed in the liver and kidney, but $CrCl_3$ caused significantly higher Cr accumulation than CrPic in the femur of rats given 100 μg Cr/g. Daily urinary Cr excretion elevated with the increase of the dietary Cr level. Rats given CrPic showed significantly higher daily urinary Cr excretion than those given $CrCl_3$, particularly at a dietary Cr level of 100 μg/g. The rate of urinary Cr excretion in rats given CrPic was constant, irrespective of the dietary Cr level, but that of rats given $CrCl_3$ fell with the increase of the dietary Cr level. These results indicate that the lowest adverse effect level of dietary Cr is less than 100 μg/g, irrespective of the chemical species of Cr.

Key words: Trivalent chromium, Chromium chloride, Chromium picolinate, Excess intake, The lowest observed adverse effect level (LOAEL)

INTRODUCTION

Trivalent chromium (Cr) is an essential trace element for human and animal nutrition (Mertz, 1993) and enhances insulin function in the form of a low molecular weight Cr binding substance (LMWCr), called chromodulin (Vincent, 2004). Severe Cr deficiency induces impaired glucose tolerance and is believed to increase the risk for diabetes (Mertz, 1969). Since a high dose (200 to 1,000 μg/day) of Cr improved glycemia among patients with diabetes (Lamson and Plaza, 2002; Rabinovitz, 2004), some healthy people take a Cr supplement, expecting an improvement of lipid or glucose metabolism. Cr chloride ($CrCl_3$), Cr nicotinate, and Cr picolinate (CrPic) are used as formulations of trivalent Cr. Among these, CrPic is designed to improve absorption (Evans and Pouchnik, 1993) and is the most common Cr supplement. In the U.S., manufactures and sellers of Cr supplements ardently promote the benefits of Cr in the prevention and treatment of type 2 diabetes, dyslipidemia, and cardiovascular disease. As a result, estimated sales of Cr supplements to consumers were about $150 million in

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2008, exceeding those of calcium supplements (Nutrition Business Journal, 2009).

In spite of the general public's acceptance of Cr supplements, the scientific basis of the protective efficacy of Cr against disease, including diabetes, is weak. In a systematic review of the effect of Cr supplementation at 200 to 1,000 μg/day on glucose metabolism and lipid levels, it was concluded that no significant effect of Cr on lipid or glucose metabolism was found in people without diabetes (Balk *et al.*, 2007). In contrast, excess intake of trivalent Cr can possibly have adverse effects on humans and animals. In fact, several case reports pointed out the occurrence of adverse effects, including anemia, liver and kidney dysfunctions, or muscle cramps due to the ingestion of CrPic at 200 to 1,000 μg Cr/day (Wasser, 1997; Martin and Fuller, 1998; Wani *et al.*, 2006). However, the possibility that other supplements or drugs ingested concomitantly are associated with these symptoms cannot be excluded in these cases and there are no available data giving clear information on the dose-response relationship between Cr and its adverse effects. Due to limited human data on the toxicity of trivalent Cr, many developed countries have not established a tolerable upper limit (UL) of Cr (Food and Nutrition Board of U.S., 2001; Scientific Committee on Food of European Commission, 2003; Ministry of Health, Labour and Welfare of Japan, 2009).

The Cr intake of Japanese adults was estimated to be about 120 μg/day (Otsuka *et al.*, 2000). Since this estimated value far exceeds 25 to 40 μg/day, which is the recommended dietary allowance of Cr for Japanese adults (Ministry of Health, Labour and Welfare of Japan, 2009), the possibility of dietary Cr deficiency in the general Japanese is extremely low. Thus, it is thought that the use of Cr supplements does not have benefits but rather adverse effects on healthy Japanese. In the present study, we examined urinary Cr excretion and Cr accumulation in the organs of weanling rats fed experimental diets containing graded levels of $CrCl_3$ or CrPic and attempted a risk assessment of the excess intake of trivalent Cr.

MATERIALS AND METHODS

Reagents

$CrCl_3$ (Cr (III) chloride hexahydrate) was purchased from Nacalai Tesque (Kyoto, Japan). CrPic (Cr (III) picolinate) was purchased from Tokyo Chemical Industry (Tokyo, Japan). The ingredients of animal diets were purchased from Oriental Yeast Co. (Tokyo, Japan). Cr standard solution (1,000 μg Cr/ml as $K_2Cr_2O_7$ in 0.1 M HNO_3), rhodium (Rh) standard (1,000 μg Rh/ml of as $Rh(NO_3)_2$ in 2 M HNO_3), distilled water (HPLC grade), and metal-free grade HNO_3 were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Animal experiments

The experimental protocol was reviewed and approved by the Animal Ethics Committee of Kansai Medical University and followed the "Guide for the Care and Use of Experimental Animals" of the Prime Minister's Office of Japan. Experimental animals were fed in a room under a controlled 12 hr light (8:00 to 20:00) and dark cycle at a temperature of 23 to 24°C and humidity of 60%. The animals were given experimental diets and deionized water *ad libitum* during the entire experimental period.

Thirty-six male weanling 4-weeks-old Wistar rats were divided into six groups and fed a casein-based semi-purified basal diet (Cr content: < 0.02 μg/g) supplemented with 1, 10, or 100 μg/g of Cr as $CrCl_3$ or CrPic. The basal diet excluded $CrCl_3$ from the AIN93G diet (Reeves *et al.*, 1993). On the 23rd to 26th day after the start of feeding, urine samples were collected from all rats. After feeding for 28 days, the rats were anesthetized with sodium pentobarbital, blood was collected from the abdominal aorta, and the liver, kidney and femur were excised, washed with saline, blotted and weighed.

A part of each blood sample was transferred to a heparinized tube and subjected to hemoglobin determination. Serum was separated from the remainder of the blood and subjected to serum biochemical tests (total protein, albumin, triglyceride, total cholesterol, glucose, urea, iron, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT)). The hemoglobin measurement and serum biochemical tests were performed by a commercial service system (Japan Medical Laboratory, Osaka, Japan).

Analysis of Cr

Cr in the organ and urine samples was determined by inductively coupled plasma mass spectrometry (ICPMS) using Rh as an internal standard. Up to 1 g of the liver, kidney or femur was heated with 5 ml of metal-free HNO_3 in a boiling water bath until the disappearance of insoluble components. The volume of the digest was made up to 25 ml with distilled water. One milliliters of urine was diluted with 9 ml of 0.1 M HNO_3. Cr in the diluted digest and urine was directly nebulized to ICPMS. Details of the ICPMS operating condition for Cr determination were described previously (Yoshida *et al.*, 2008).

Histopathology

Liver samples were sectioned and the blocks obtained

were fixed in 4% formaldehyde. Tissues were embedded in paraffin, sectioned at a thickness of 5 μm, and stained with hematoxylin and eosin. A pathologist without knowledge of the animal treatment evaluated the sections.

Statistics

Experimental data were assessed by two-way analysis of variance (ANOVA). When the F value was significant ($p < 0.05$) in the Cr level, the Scheffe multiple range test was performed to determine which pairs of the Cr levels were significantly different ($p < 0.05$). In addition, when the F value was significant ($p < 0.05$) in the interaction between the Cr level and Cr species, the Scheffe multiple range test was performed to determine which pairs of the means were significantly different ($p < 0.05$). These statistical tests were performed using a personal computer (eMac; Apple Computer, Cupertino, CA, USA) with operating system Mac OS 9.2 and statistical program package StatView-J version 5.0 (Abacus Concept, Berkeley, CA, USA).

RESULTS

Rat growth and organ weights

Table 1 shows the body, liver and kidney weights of rats fed experimental diets containing 1 to 100 μg/g of Cr as $CrCl_3$ or CrPic for 28 days. Among the experimental groups, no significant difference was observed in the body weight; however, supplementation of 100 μg Cr/g to the diets caused a significantly lower liver weight. In the kidney weight, rats fed diet containing 100 μg Cr/g as CrPic tended to show lower values compared to other groups of rats.

Serum biochemical tests and histopathology of liver

Table 2 shows serum AST and ALT activities in rats fed experimental diets. Dietary supplementation with CrPic at a level of 100 μg Cr/g significantly elevated these serum transaminase activities. In spite of the elevation of AST and ALT activities, no hepatic histopathological changes were observed even in rats fed a diet containing CrPic at 100 μg Cr/g.

No significant difference was observed among experimental groups in the hemoglobin level and serum biochemical tests, except for transaminase activities.

Cr in organs

Table 3 summarizes Cr concentration in the liver, kidney and femur. In all organs examined, Cr accumulation increased dependently on the dietary Cr level. No influence of differences in the chemical form of supplemented Cr was observed in the liver and kidney, but was manifested in the femur; $CrCl_3$ caused significantly higher Cr accumulation than CrPic in the femur of rats fed diets containing 100 μg Cr/g.

Urinary Cr excretion

Table 4 shows the urinary Cr excretion of rats fed the experimental diets. Daily urinary Cr excretion elevated with the increase of the dietary Cr level. Differences in the chemical form of dietary Cr marked influenced urinary Cr excretion. Rats given CrPic showed significantly higher daily urinary Cr excretion than those given $CrCl_3$, particularly at a dietary Cr level of 100 μg/g. In rats given CrPic, the rate of urinary Cr excretion was constant (1.36 to 1.52%) irrespective of the dietary Cr level. On the other hand, the rate of urinary excretion fell with increased dietary Cr in rats given $CrCl_3$.

DISCUSSION

In the present study, dietary Cr supplementation decreased liver weight at 100 μg/g, irrespective of the chemical species. Since a difference between $CrCl_3$- and CrPic-loading was not observed in the hepatic Cr accumulation, the decrease must be associated with the accumulated Cr. Because no histopathological changes were detected, the decrease may not be a disease change. Nevertheless, it can be said that trivalent Cr inhibits liver growth.

In addition to the lowered liver weight, rats fed diets supplemented with CrPic at 100 μg Cr/g showed elevated serum transaminase activities. It has been reported that CrPic causes liver dysfunction in human and animals (Cerulli *et al.*, 1998; Bagchi *et al.*, 2002), and picolinate induces apoptosis in several types of cultured cells (Ogata *et al.*, 2000). Thus, it can be concluded that CrPic impairs liver cells by a mechanism different from $CrCl_3$.

Cr accumulation in the organs and urinary Cr excretion rose with increased dietary Cr and no difference with the chemical form of supplemented Cr was observed in hepatic and renal Cr accumulation. However, administration of CrPic caused lower femur Cr accumulation and higher urinary excretion than that of $CrCl_3$ at a dietary Cr level of 100 μg/g. On the other hand, while the rate of urinary Cr excretion of rats given CrPic was constant irrespective of the dietary Cr level, that of rats given $CrCl_3$ decreased with the increase of the dietary Cr level. In several organs, LMWCr is estimated to exist as the apo form (Yamamoto *et al.*, 1987). After the intake of a large dose of Cr, apoLMWCr probably binds to Cr and carries it into

Table 1. Effect of dietary Cr administration on body, liver and kidney weights of growing rats

Dietary Cr level (μg/g)	Body weight (g)		Liver weight (g/100 g body weight)		Kidney weight (g/100 g body weight)	
	$CrCl_3$	CrPic	$CrCl_3$	CrPic	$CrCl_3$	CrPic
1	246 ± 5	243 ± 5	4.63 ± 0.16	4.71 ± 0.15	0.79 ± 0.01	0.78 ± 0.03
10	252 ± 8	242 ± 5	4.46 ± 0.10	4.59 ± 0.20	0.81 ± 0.03	0.80 ± 0.02
100	246 ± 3	244 ± 4	4.14 ± 0.09	4.01 ± 0.08	0.78 ± 0.04	0.71 ± 0.02
Two-way ANOVA followed by the Scheffe multiple range test						
$CrCl_3$ *vs.* CrPic	NS		NS		NS	
Cr level	NS		$p < 0.001$		NS	
1 μg/g *vs.* 10 μg/g	-		NS		-	
1 μg/g *vs.* 100 μg/g	-		$p < 0.001$		-	
10 μg/g *vs.* 100 μg/g	-		$p = 0.009$		-	
Interaction	NS		NS		NS	

Means ± S.E.M. (n = 6).

Table 2. Effect of dietary Cr administration on serum AST and ALT activities in growing rats

Dietary Cr level (μg/g)	AST (IU/ml)		ALT (IU/ml)	
	$CrCl_3$	CrPic	$CrCl_3$	CrPic
1	102 ± 3[a]	107 ± 2[a]	43 ± 4[a]	39 ± 3[a]
10	83 ± 6[a]	102 ± 4[a]	42 ± 3[a]	43 ± 3[a]
100	95 ± 3[a]	179 ± 19[b]	35 ± 3[a]	63 ± 4[b]
Two-way ANOVA				
$CrCl_3$ *vs.* CrPic	$p < 0.001$		$p = 0.004$	
Cr level	$p < 0.001$		$p = 0.030$	
Interaction	$p < 0.001$		$p < 0.001$	

Means ± S.E.M. (n = 6). Values in the same parameter not sharing a common superscript differ significantly ($p < 0.05$).

the urine (Wada *et al.*, 1983). Accordingly, the decreased urinary excretion rate of Cr in rats given large amounts of $CrCl_3$ indicates that excess Cr saturates the urinary Cr excretion process *via* LMWCr. Thus, not Cr absorption but Cr excretion is thought to be higher in rats given CrPic than given $CrCl_3$. Picolinic acid increases urinary excretion as well as absorption of zinc and results in the increased turnover of endogenous zinc (Seal and Heaton, 1985). Similarly to zinc, Cr excretion may have been accelerated by picolinate, resulting in low Cr accumulation in the femur.

Anderson *et al.* (1997) observed no change of body, liver and kidney weights as well as serum biochemical tests, including transaminase activities in weanling rats fed diets supplemented with $CrCl_3$ or CrPic up to 100 μg Cr/g, and emphasized the lack of toxicity of trivalent Cr. In addition, they observed that rats consuming CrPic-supplemented diets had several-fold higher Cr concentrations in both the liver and kidney than those fed $CrCl_3$ and concluded that the absorption of CrPic was higher than $CrCl_3$. The reason for the inconsistency between the studies of Anderson *et al.* (1997) and ours could not be sufficiently

Table 3. Cr concentration in organs of growing rats fed experimental diets containing graded level of $CrCl_3$ or CrPic

Dietary Cr level (μg/g)	Liver (μg/g)		Kidney (μg/g)		Femur (μg/g)	
	$CrCl_3$	CrPic	$CrCl_3$	CrPic	$CrCl_3$	CrPic
1	0.46 ± 0.11	0.31 ± 0.03	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.01[a]	0.03 ± 0.01[a]
10	0.87 ± 0.01	0.85 ± 0.04	0.12 ± 0.01	0.11 ± 0.01	0.16 ± 0.02[a]	0.17 ± 0.01[a]
100	1.86 ± 0.21	1.96 ± 0.24	0.81 ± 0.22	1.00 ± 0.11	1.79 ± 0.20[c]	0.61 ± 0.06[b]
Two-way ANOVA followed by the Scheffe multiple range test						
$CrCl_3$ *vs.* CrPic	NS		NS		$p < 0.001$	
Cr level	$p < 0.001$		$p < 0.001$		$p < 0.001$	
1 μg/g *vs.* 10 μg/g	$p = 0.007$		NS		NS	
1 μg/g *vs.* 100 μg/g	$p < 0.001$		$p < 0.001$		$p < 0.001$	
10 μg/g *vs.* 100 μg/g	$p < 0.001$		$p < 0.001$		$p < 0.001$	
Interaction	NS		NS		$p < 0.001$	

Means ± S.E.M. (n = 6). Values in the femur not sharing a common superscript differ significantly ($p < 0.05$).

Table 4. Urinary Cr excretion of growing rats fed experimental diets containing graded level of $CrCl_3$ or CrPic

Dietary Cr level (μg/g)	Excretion amounts (μg/day)		Excretion rate (%)	
	$CrCl_3$	CrPic	$CrCl_3$	CrPic
1	0.41 ± 0.03[a]	0.40 ± 0.02[a]	1.65 ± 0.12[b]	1.47 ± 0.09[b]
10	2.89 ± 0.42[a]	3.71 ± 0.48[a]	1.15 ± 0.17[ab]	1.52 ± 0.16[b]
100	14.65 ± 0.99[b]	33.35 ± 5.69[c]	0.59 ± 0.04[a]	1.36 ± 0.22[b]
Two-way ANOVA				
$CrCl_3$ *vs.* CrPic	$p = 0.002$		$p = 0.013$	
Cr level	$p < 0.001$		$p = 0.002$	
Interaction	$p < 0.001$		$p = 0.011$	

Means ± S.E.M. (n = 6). Excretion rate was calculated as follows: (excretion amounts)/[(dietary Cr level) × (feed intake)] × 100. Values in the same parameter not sharing a common superscript differ significantly ($p < 0.05$).

clarified. Differences between the two studies exist in the strain of rats, the feeding period and the basal diet used. Among these, differences in the basal diet may influence the manifestation of Cr toxicity and Cr metabolism. In the present study, we used a standard semi-purified diet, called AIN93G diet, as the basal diet. This diet is composed of purified casein and starch, sucrose, soybean oil, and mineral and vitamin mixtures (Reeves *et al.*, 1993). On the other hand, Anderson *et al.* (1997) used a commercial stock diet (RMH 3200, Agway Inc., Waverly, NY, USA). This diet is composed of non-purified feed materials, such as cereal and grass, and contains various fibers and flavonoids. Since these substances are known to inhibit the intestinal absorption of several kinds of metals (Hurrell *et al.*, 1999; Sandberg, 2002; Zijp *et al.*, 2000) and prevent liver impairment (Ibrahim *et al.*, 2008; Park *et al.*, 2008), intake of the stock diet may have modified Cr absorption and CrPic-induced liver impairment. However, because raw materials and composition of these commercial stock diets including the RMH 3200 are not

constant, it is impossible to evaluate Cr toxicity under the same condition as the Anderson's study. Nevertheless, it is necessary to examine the effect of difference in basal diet (stock diets or semi-purified diets) on the manifestation of Cr toxicity.

A general human diet is not a semi-purified diet, but it is impossible to evaluate the effects of numerous unknown components contained in the human diets on Cr toxicity. Accordingly, due to the least unknown dietary factor, the results on Cr toxicity, tissue accumulation and urinary excretion obtained from our study using a semi-purified diet are available for a risk assessment of trivalent Cr. As for CrPic, since a dietary level of 100 μg Cr/g decreased the liver weight and elevated serum transaminase activities, the lowest observed adverse effect level (LOAEL) in the diet can be estimated to be below 100 μg Cr/g. Similarly, the LOAEL for dietary $CrCl_3$ was also below 100 μg Cr/g since saturation of the urinary Cr excretion process was observed at this dose level. Thus, it is concluded that the LOAEL for dietary Cr is less than 100 μg/g, irrespective of the chemical species of Cr. Since rats eat about 10% of their body weight every day, the dietary level of 100 μg/g corresponded to about 10 mg/kg. However, it is known that trivalent Cr shows mutagenicity or damages chromosomes in animal cells (Stearns *et al.*, 1995; 2002); therefore, research extend over the second generation is necessary for the risk assessment of trivalent Cr.

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REFERENCES

Anderson, R.A., Bryden, N.A. and Polansky, M.M. (1997): Lack of toxicity of chromium chloride and chromium picolinate in rats. J. Am. Coll. Nutr., **16**, 273-279.

Bagchi, D., Stohs, S.J., Downs, B.W., Bagchi, M. and Preuss, H.G. (2002): Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology, **180**, 5-22.

Balk, E.M., Tatsioni, A., Lichtenstein, A.H., Lau, J. and Pittas, A.G. (2007): Effect of chromium supplementation on glucose metabolism and lipids: a systematic review of randomized controlled trials. Diabetes Care, **30**, 2154-2163.

Cerulli, J., Grabe, D.W., Gauthier, I., Malone, M. and McGoldrick, M.D. (1998): Chromium picolinate toxicity. Ann. Pharmacother., **32**, 428-431.

Evans, G.W. and Pouchnik, D.J. (1993): Composition and biological activity of chromium-pyridine carboxylate complexes. J. Inorg. Biochem., **49**, 177-187.

Food and Nutrition Board, Institute of medicine, National Academy of Sciences (2001): Chromium. In Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. pp.197-223, National Academy Press, Washington DC.

Hurrell, R.F., Reddy, M. and Cook, J.D. (1999): Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. Br. J. Nutr., **81**, 289-295.

Ibrahim, W.H., Habib, H.M., Chow, C.K. and Bruckner, G.G. (2008): Isoflavone-rich soy isolate reduces lipid peroxidation in mouse liver. Int. J. Vitam. Nutr. Res., **78**, 217-222.

Lamson, D.W. and Plaza, S.M. (2002): The safety and efficacy of high-dose chromium. Altern. Med. Rev., **7**, 218-235.

Martin, W.R. and Fuller, R.E. (1998): Suspected chromium picolinate-induced rhabdomyolysis. Pharmacotherapy, **18**, 860-862.

Mertz, W.H. (1969): Chromium occurrence and function in biological systems. Physiol. Rev., **49**, 163-239.

Mertz, W.H. (1993): Chromium in human nutrition: A review. J. Nutr., **123**, 626-633.

Ministry of Health, Labour, and Welfare (2009): Dietary Reference Intakes for Japanese 2010, pp.218-275, Dai-ichi shuppan, Tokyo.

Nutrition Business Journal (2009): NBJ's Supplement Business Report 2009, pp.169-188, Penton Media Inc., San Diego.

Ogata, S., Inoue, K., Iwata, K., Okumura, K. and Taguchi, H. (2001): Apoptosis induced by picolinic acid-related compounds in HL-60 cells. Biosci. Biotechnol. Biochem., **65**, 2337-2339.

Otsuka, Y., Isomoto, S. and Noda, H. (2000): Dietary intake of trace elements in the general population, estimated from a regional nutritional survey, and comparison with recommended dietary allowances and tolerable upper intake levels. Nippon Koshu Eisei Zasshi (Jpn. J. Public Health), **47**, 809-818 (in Japanese).

Park, S.W., Lee, C.H., Kim, Y.S., Kang, S.S., Jeon, S.J., Son, K.H. and Lee, S.M. (2008): Protective effect of baicalin against carbon tetrachloride-induced acute hepatic injury in mice. J. Pharmacol. Sci., **106**, 136-143.

Rabinovitz, H., Friedensohn, A., Leibovitz, A., Gabay, G., Rocas, C. and Habot, B. (2004): Effect of chromium supplementation on blood glucose and lipid levels in type 2 diabetes mellitus elderly patients. Int. J. Vit. Nutr. Res., **74**, 178-182.

Reeves, P.G., Nielsen, F.H. and Fahey, G.C. (1993): AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J. Nutr., **123**, 1939-1951.

Sandberg, A.S. (2002): Bioavailability of minerals in legumes. Br. J. Nutr., **88**, S281-285.

Scientific Committee on Food, European Commission (2003): Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Trivalent Chromium, European Commission, Brussels (expressed on 4 April 2003).

Seal, C.J. and Heaton, F.W. (1985): Effect of dietary picolinic acid on the metabolism of exogenous and endogenous zinc in the rat. J. Nutr., **115**, 986-993.

Stearns, D.M., Wise, J.P.Sr., Patierno, S.R. and Wetterhahn, K.E. (1995): Chromium(III) picolinate produces chromosome damage in Chinese hamster ovary cells. FASEB J., **9**, 1643-1648.

Stearns, D.M., Silveira, S.M., Wolf, K.K. and Luke, A.M. (2002):

Chromium(III) tris(picolinate) is mutagenic at the hypoxanthine (guanine) phosphoribosyltransferase locus in Chinese hamster ovary cells. Mutat. Res., **513**, 135-142.

Vincent, J.B. (2004): Recent advances in the nutritional biochemistry of trivalent chromium. Proc. Nutr. Soc., **63**, 41-47.

Wada, O., Wu, G.Y., Yamamoto, A., Manabe, S. and Ono, T. (1983): Purification and chromium-excretory function of low-molecular-weight, chromium-binding substances from dog liver. Environ. Res., **32**, 228-239.

Wani, S., Weskamp, C., Marple, J. and Spry L. (2006): Acute tubular necrosis associated with chromium picolinate-containing dietary supplement. Ann. Pharmacother., **40**, 563-566.

Wasser, W.G., Feldman, N.S. and D'Agati, V.D. (1997): Chronic renal failure after ingestion of over-the-counter chromium picolinate. Ann. Intern. Med., **126**, 410.

Yamamoto, A., Wada, O. and Ono, T. (1987): Isolation of a biologically active low-molecular-mass chromium compound from rabbit liver. Eur. J. Biochem., **165**, 627-631.

Yoshida, M., Takada, A., Hirose, J., Endô, M., Fukuwatari, T. and Shibata, K. (2008): Molybdenum and chromium concentrations in breast milk from Japanese women. Biosci. Biotechnol. Biochem., **72**, 2247-2250.

Zijp, I.M., Korver, O. and Tijburg, L.B. (2000): Effect of tea and other dietary factors on iron absorption. Crit. Rev. Food Sci. Nutr., **40**. 371-398.

特集「日本人の食事摂取基準(2010年版)」

日本人の微量ミネラルの食事摂取基準(2010)

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Dietary Reference Intakes of Trace Minerals for Japanese (2010)

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Key words: Japanese, dietary reference intake, iron, zinc, copper, manganese, iodine, selenium, chromium, molybdenum

はじめに

日本人の食事摂取基準2010年版におけるミネラルの区分は,「多量ミネラル(ナトリウム,カリウム,カルシウム,マグネシウム,リン)」と「微量ミネラル(鉄,亜鉛,銅,マンガン,ヨウ素,セレン,クロム,モリブデン)」である.本稿では「微量ミネラル」に関して,その呼称,範囲,順序を決めた経緯を概説し,次いで各微量ミネラルの食事摂取基準策定の根拠と今後の課題について述べる.なお,各微量ミネラルの食事摂取基準の詳細は,厚生労働省「日本人の食事摂取基準」策定検討会報告書[1]もしくは他誌に記載されている解説[2]を参照していただきたい.

1. 呼称,範囲,順序

食事摂取基準2005年版では鉄以下の8元素を「微量元素」と呼んでいた.これはtrace elementsを邦訳したものであり,学術的には誤りでない.しかし,医学・生物学分野における微量元素には,水銀やカドミウムなど,ヒトにおける非必須元素も含まれている.食事摂取基準で扱う無機元素は栄養上必須のものに限定していることから,栄養学分野で馴染み深い「ミネラル」という用語を「元素」と置き換え,「微量ミネラル」という呼称を採用した.また,これに合わせて,ナトリウム,カリウム,カルシウム,マグネシウム,リンについても,「多量ミネラル」としてまとめることとした.

摂取基準で扱う微量ミネラルの範囲についても食事摂取基準策定検討会ミネラルワーキンググループにおいて検討した.とくにフッ素について,う歯予防の観点から食事摂取基準を策定することの是非を検討した.しかし,フッ素はヒトにおいて欠乏症は認められておらず栄養学上の必須元素ではない.しかも,う歯予防の効果を得るに必要と推定される摂取量は日常の食事から摂取できる量を大幅に超えている.これらの理由により,フッ素は食事摂取基準が扱う微量ミネラルの範囲には含めないこととした.

報告書におけるミネラルの掲載順序についても検討した.当初は原子番号,アルファベット,アイウエオなど,機械的に順序を決める案が有力であったが,現実の献立作成での優先度,および食品成分表や国民健康・栄養調査における掲載順序を考慮し,微量ミネラルに関しては,鉄,亜鉛,銅,マンガン,ヨウ素,セレン,クロム,モリブデンの順にすることとした.今後,栄養学のテキストにおいて,微量ミネラルという呼称とこの順序が定着することを期待している.

2. 鉄の食事摂取基準

鉄の食事摂取基準を付録(p249参照)に示した.鉄の食事摂取基準は,2005年版と同様に,米国/カナダの食事摂取基準(以下,米国と略記)[3]でも採用されている要因加算法にもとづいて策定した.別の方法,とくに集団データ解析法についても検討したが,日本人では,鉄欠乏の指標とされる血清フェリチン濃度が食事からの鉄摂取量

以外の要因に依存して変動することが報告されていることから[4]，今回の策定においても要因加算法を採用することとした．

要因加算法における要因とは，基本的損失(大半は消化管排泄)，月経血への損失，成長に伴う蓄積(大半は血液量増加によるヘモグロビンの増加)，妊娠・授乳に伴う需要増加，および消化管吸収率である．すなわち，鉄の推定平均必要量は，「(基本的損失＋経血損失(思春期以降の閉経前女性のみ)＋妊娠・授乳に伴う需要増加(妊娠・授乳期のみ)＋成長に伴う蓄積(成長期のみ))÷消化管吸収率」の式を用いて算定し，推奨量は推定平均必要量に1.2(成長期は1.4)を乗じて策定した．いずれの要因についても，鉄栄養が健全と推定されるヒトを対象とした内外の研究から妥当と思われる数値を採用した．2005年版と比較した場合，妊婦付加量以外は，基準体位の変化に伴う軽微な変更が生じたのみである．ただし，経血損失量と消化管吸収率に関しては，以下に述べるように，日本人を対象にした詳細な研究が今後必要と思われる．

今回の策定では，18歳以上の経血量として37 mL/回，月経周期31日を採用した[5]．しかし，これらの数値の根拠となる日本人の経血量についての研究は，きわめて例数が少なく，かつ対象としているのはいずれも小地域の特定集団である[6][7]．しかも中には1960年代に実施されたものもある．半世紀前と現在とでは女性の体位も変化し，かつ生活環境が大きく異なっていることから，現代女性を対象とした大規模な経血に関する調査が行われることを期待する．

同様に，鉄の消化管吸収率についても日本人を対象にした研究が見当たらないため，欧米の研究にもとづきFAO/WHOが指示している15%を用いた[8]．しかし，食事中の鉄の吸収率は，鉄の形態，共存成分，鉄摂取量に伴い変化することから，日本人が日常的な献立を摂取した場合の数値を求める必要がある．

妊娠中の鉄付加量は，妊娠に伴う鉄需要増加(胎児・胎盤・臍帯への鉄蓄積と妊娠に伴う循環血液量増加)をもとに算定できる．2005年版においてはWHOの需要量増加推定値[9]を採用したが，2010年版では日本人の体格をもとに循環血液量増加を少なく見積もったので付加量は小さな数値になった．それでも妊娠中期以降(18～29歳)の付加量を加えた推定平均必要量と推奨量は，それぞれ17.5 mg/日と21.0 mg/日となり，一般的な献立からは達成が困難な数値となる．しかし，現実には，妊娠女性の貧血有病率は一般女性よりもわずかに高い程度である[10]．この矛盾は，おそらく妊娠中の鉄需要増加に伴う鉄吸収率の増加がきわめて大きいことに起因していると推定できる．そこで，日本人の妊娠女性の鉄吸収率を40%[11]と高めに見積もった場合の妊娠中期以降の付加量を推定平均必要量8.0 mg/日，推奨量9.5 mg/日と試算した．この試算値にもとづけば，妊娠中期以降(18～29歳)の鉄摂取の付加量を加えた推定平均必要量と推奨量は，それぞれ13.0 mg/日と15.5 mg/日となる．吸収率40%の科学的根拠が低いため，これらの試算値は，食事摂取基準としては採用できなかったが，妊娠女性の鉄摂取の現実的な目標といえるだろう．

なお耐容上限量に関しては，新規な報告が見当たらないため2005年版同様に，FAO/WHOの暫定耐容最大1日摂取量0.8 mg/kg[12]にもとづき策定した．ヒトを対象にして上限量策定に有用な実験を行うことは倫理的に難しい．しかし，多種多様な鉄サプリメントや鉄強化食品が市販されていることから，鉄を大量摂取している症例が存在している可能性は高い．このような症例は，健康障害が認められなくても，上限量策定にとっては有用であることから，積極的に報告されることを希望するものである．

3. 亜鉛の食事摂取基準

亜鉛の食事摂取基準を付録(p249参照)に示した．亜鉛の食事摂取基準も2005年版と同様に，米国[13]でも採用されている要因加算法で求めた．すなわち，「総排泄量＝腸管内因性排泄量＋尿中排泄量＋体表消失量＋精液または月経血への損失量」と考え，総排泄量に見合う真の吸収量を与える摂取量を「真の吸収量＝1.113×摂取量$^{0.5462}$」の式より算定し，男性11.18 mg/日，女性10.03 mg/日という数値を得た．要因中でもっとも寄与の大きい腸管内因性排泄量を求める関係式は男性を対象とした英米の複数の研究から得られるが，これらの研究における対象者の体重は特定できなかった．そこで，この腸管内因性排泄量は米国人男性の基準体位である76 kgの人に対するものであると考え，最終的に得られた上記摂取量を76 kgの人に対する推定平均必要量とした．そして18歳以上は体重比の0.75乗，12～17歳は体重比の0.75乗と成長因子を用いて外挿し，性・年齢階級別の推定平均必要量を策定した．1～11歳に関しては，日本人小児を対象とした出納試験における平衡維持量[14]と米国人の値から推定した小児の体表損失量にもとづいて推定平均必要量を策定した．推奨量は推定平均必要量に1.2を乗じて求めた．2005年版では総排泄量を求めるための要因の一部に体重の小さい日本人の数値を用いていた．このため2010年版において，とくに成人の数値は男女ともに2005年版に比較して大きな数値になった．

以上から明らかなように，亜鉛の食事摂取基準策定の根拠となっている研究は，そのほとんどが欧米のものである．最大の寄与要因である腸管内因性排泄量に関して，日本人を対象にして安定同位体を用いた研究が待たれる．

亜鉛の耐容上限量は，鉄と同様に新規な報告が見当たらないため，2005年版において採用した米国の成人女性を対象とした研究から得られる最低健康障害発現量60 mg/日[15]と不確実性因子1.5にもとづいて策定した．ただし各年齢階級への外挿においては，2005年版とは異な

り，男女ともに年齢階級別基準体重の 61 kg（米国成人女性の基準体重）に対する比を用いた．このため 2010 年版の耐容上限量は，男性において 2005 年版よりも大きな数値になった．亜鉛サプリメントや亜鉛強化食品が市販されていることから，今後は亜鉛大量摂取の症例を積極的に報告することが必要といえる．

4. 銅の食事摂取基準

銅の食事摂取基準を付録（p249 参照）に示した．銅の摂取基準は，銅の栄養状態を示すバイオマーカー（血漿銅濃度，血漿スーパーオキシドジスムターゼ活性など）の値が低下しない最小の摂取量（0.72 mg/ 日）[16)17)] にもとづき策定した．この方法は 2005 年版と同じであり，米国 [18)] も採用している．したがって 2010 年版の数値は，2005 年版に比較して基準体位の変化に伴う軽微な変更のみである．2005 年版発表直後は，推奨量が 6 次改定栄養所要量の約半分の数値になったため，相当な論議を呼んだが，この 5 年の間に上記 0.72 mg/ 日を推定平均必要量策定の基準値にすることを支持する報告 [19)] が提出されており，今後もよほどのことがない限り，大きな変更はないと思われる．耐容上限量に関しても，新規な報告が見当たらないため，2005 年版をそのまま踏襲した．

5. マンガンの食事摂取基準

マンガンの食事摂取基準を付録（p249 参照）に示した．マンガンの食事摂取基準に関しては，2005 年版と同様に，日本人のマンガン摂取量にもとづいて目安量を設定するにとどめた．これは，短期間の出納実験から求められるマンガンの平衡維持量の信頼性を米国同様に低いと判断しているためである．日本人のマンガン摂取量に関する報告も増えていないので，2005 年版と同様に，複数の報告値に基づいて成人日本人の平均的なマンガン摂取量を 3.7 mg/ 日と見積もった [20)]．そして，エネルギー摂取量の性差を考慮し，男性 4.0 mg/ 日，女性 3.5 mg/ 日を 18 歳以上の目安量とした．なお，国民健康・栄養調査の元データと食品成分表に付記されている食品中マンガン濃度にもとづいて成人の性・年齢階級別マンガン摂取量を試算したところ，上記目安量は，日本人のマンガン摂取量の中央値にほぼ一致していた．マンガンは穀物をはじめとする植物性食品に多く含有されている．このため日本人のマンガン摂取量は米国人の約 1.5 倍である．したがって日本人のマンガン摂取の目安量も米国の目安量 [21)] の約 1.5 倍となっている．

以上のように，マンガンは，微量ミネラル中で唯一，推定平均必要量と推奨量が策定できなかった．マンガンは，欠乏症もほとんど認められず，研究者の関心を引きにくい微量栄養素かもしれない．しかし，推定平均必要量策定に根拠を与える質の高い研究が国内の研究者によって行われることを期待している．

6. ヨウ素の食事摂取基準

ヨウ素の食事摂取基準を付録（p249 参照）に示した．ヨウ素の食事摂取基準は，2005 年版に比較して，推定平均必要量と推奨量は数値の丸め方に伴う軽微な変更であったのに対して，耐容上限量は大きな変更が加わった．ここでは耐容上限量について述べる．

米国 [22)] では，健常人（ヨウ素摂取量約 300 μg/ 日）へ 1500 μg/ 日のヨウ素を負荷した場合に甲状腺機能低下が起こること [23)] から，上限量を 1100 μg/ 日としている．2005 年版では，一般的な日本人のヨウ素摂取は最大で 3000 μg/ 日と推定できるが [24)25)]，ヨウ素の過剰摂取に起因する甲状腺機能低下が認められないこと，および北海道住民を対象にした研究 [26)] から甲状腺機能低下が生じるヨウ素摂取量は 10 mg/ 日であると判断できることから，上限量を 3000 μg/ 日としていた．しかし，最近に行われた，中国 [27)]，およびアフリカ [28)] における研究は，継続的な 1500 μg/ 日程度のヨウ素摂取が甲状腺腫の有病率を上昇させることを示しており，日本人のヨウ素摂取と甲状腺機能の関連について再検討する必要があると判断された．

まず，日本人のヨウ素摂取量を尿中ヨウ素排泄量 [29)]，および昆布の消費量 [30)] の両面から検討し，日本人のヨウ素摂取量は，日常的には 500 μg/ 日未満であるが，間欠的に海藻類を大量に摂取するために平均的には約 1500 μg/ 日になると推定した．次に，2005 年版において上限量策定の根拠とした北海道住民を対象とした疫学研究 [26)31)] を再検討し，成人日本人におけるヨウ素の健康障害非発現量を 3300 μg/ 日と推定した．そして，諸外国で行われているヨウ素過剰障害に関する研究に配慮し，安全性を高める観点から不確実性因子を 1.5 と見積もり，18 歳以上の耐容上限量を 2005 年版よりも 800 μg/ 日小さい 2200 μg/ 日とした．

一方，ヨウ素摂取が約 750 μg/ 日である北海道の小学生において甲状腺容積の有意な増大が認められており，小児ではヨウ素摂取が 500 μg/ 日を超えると有害な影響が生じると考えられている [32)]．これにもとづき，6 ～ 11 歳の耐容上限量を 500 μg/ 日とし，他の性・年齢階級にはこの値と成人の値から体重比で外挿した数値を適用した．また，ヨウ素過剰摂取と推定される乳児のヨウ素摂取量 [33)] にもとづき，乳児の耐容上限量は 250 μg/ 日とした．なお，これらの 18 歳未満に対するヨウ素の耐容上限量は 2010 年版において初めて策定したものである．

ヨウ素の耐容上限量の策定において痛感したことは，日本人のヨウ素摂取量を推定している論文が意外に少ないということであった．日本人が本当に米国の耐容上限量を上回るヨウ素を摂取しているのであれば，なぜ過剰障害が起こらないのかを真剣に検討すべきである．その場合，ゴイトロゲン（造甲状腺腫物質）として知られるイソフラボンを含有する大豆製品がヨウ素の影響をどの程

度修飾しているかも検討課題と思う．国内におけるヨウ素の医学・生物学的研究の多くは，内分泌学や小児科学の臨床系研究者に委ねられている．これらの研究者と分析学者，および栄養学者が一同に会して，ヨウ素の耐容上限量について議論することが必要と感じている．

7. セレンの食事摂取基準

セレンの食事摂取基準を付録(p249参照)に示した．セレンの推定平均必要量は，2005年版と同様に，克山病のようなセレン欠乏症を予防するのに必要なセレン摂取量という観点から，血漿の含セレン酵素であるグルタチオンペルオキシダーゼ(GPX)の活性が飽和値の3分の2の値を示すときのセレン摂取量にもとづき策定した．この考え方は，セレン欠乏症の予防には血漿GPX活性が飽和値の3分の2の値で十分とするWHOの報告[34]にもとづくものである．米国[35]などでは，推定平均必要量を血漿GPX活性をちょうど飽和させるセレン摂取量としているため，わが国の策定値は，欧米に比べると低い水準にある．

一方，セレンの耐容上限量は，2005年版に大きな変更を加えた．2005年版では，米国[35]と同様に，慢性セレン中毒症状を指標にしたセレンの健康障害非発現量(13.3 μg/kg/日)[36]と不確実性因子2を用いて上限量を設定していた．しかし，2010年版では，米国において，血清セレン濃度が121.6 μg/L(セレン摂取量84 μg/日に相当)以上の集団に200 μg/日のセレンをセレン酵母サプリメントとして投与すると2型糖尿病の発生率が有意に上昇したと報告されたことから[37]，セレン摂取量が100 μg/日に近い人が200 μg/日のセレンをサプリメントから付加的に摂取し続けることは健康に対して好ましくない影響を与える可能性があると判断した．そこで，性・年齢階級別体重が最大である30～49歳男性(基準体重68.5 kg)の耐容上限量を300 μg/日とし，他の年齢階級には300 μg/68.5 kg/日＝4.4 μg/kg/日を適用した．なお，この4.4 μg/kg/日という数値は，慢性セレン中毒を指標にした場合の健康障害非発現量に不確実性因子3を適用したのと結果的に同じであるので，2010年版の耐容上限量は2005年版のほぼ3分の2の値となった．

低セレン摂取がいくつかのがんの発生リスクを高めるという報告は多い．これらの研究の多くは，対象者を血清セレン濃度などを指標に複数の集団に分割し，がんの発生リスクを集団間で比較している．しかし，セレン摂取とがん発生率に有意な関連が認められるのは，対象者全体のセレン栄養状態が低いときのみである[38]．たとえば，有意な関連ありとする一報告[39]では，もっとも高いセレン栄養状態である集団の血清セレン濃度は78 μg/L(セレン摂取量54 μg/日に相当)以上であるのに対して，関連なしとする別の報告[40]でのもっとも低いセレン栄養状態である集団の血清セレン濃度は99 μg/L(セレン摂取量69 μg/日に相当)未満である．これらの研究結果にもとづき，がん予防に適切なセレン摂取量を目標量として定めることも検討したが，科学的証拠が小さく，時期尚早と判断した．

8. クロムの食事摂取基準

クロムの食事摂取基準を付録(p249参照)に示した．米国ではクロムの食事摂取基準として，クロム摂取量にもとづいて目安量を策定している[41]．しかし，わが国では，食品中クロムに関して信頼性の高い分析値の報告が少なく，クロム摂取量を正確に見積もることが不可能である．このため，2010年版においても，2005年版と同様に，外国の高齢者を対象とした出納実験[42][43]の結果にもとづき，推定平均必要量を策定した．したがって，2010年版の推定平均必要量と推奨量は，2005年版に基準体位の変化に伴う軽微な変更が加わっただけである．

糖尿病患者に200～1000 μg/日のクロムサプリメントを投与すると症状の改善が認められる．しかし，健康な人へのクロムサプリメント投与が健康にとって好ましい影響を与えることは認められていない[44]．一方，200～1000 μg/日のクロムサプリメント継続摂取による副作用の報告が散発的に認められるが[45]，いずれも同時に服用していた医薬品やサプリメント類の影響を否定できない．以上より，クロム摂取と健康障害との量・反応関係に関する研究は不十分と判断し，2005年版と同様にクロムの耐容上限量設定は見合わせた．しかし，このことが200～1000 μg/日のクロム摂取が無害であることを保証するものではないことを強調したい．

なお，乳児の目安量に関して，日本人の母乳中濃度に関する報告[46]が新たに提出されたため，2010年版において初めてこれを策定した．

9. モリブデンの食事摂取基準

モリブデンの食事摂取基準を付録(p249参照)に示した．モリブデンの食事摂取基準は，2005年版に比較して，推定平均必要量と推奨量は基準値から各性・年齢層への外挿法の統一に伴う軽微な変更であったのに対して，耐容上限量は大きな変更が加わった．ここでは耐容上限量について述べる．

2005年版ではアルメニアで発生した事例[47]にもとづいて，モリブデンの耐容上限量を5 μg/kg/日(体重60 kgのヒトで300 μg/日)としていた．しかし，この事例報告には多くの問題点があり，記載されている所見にモリブデンが関わることは疑わしいと判断した．一方，日本人のモリブデン摂取量を150～350 μg/日と見積もる報告[48][49]が提出され，日本人にモリブデン過剰摂取に伴う健康障害の報告がないことから，2005年版の上限量は厳し過ぎるという指摘もなされていた．欧米の食事摂取基準[50][51]では，ラットにおけるモリブデンの健康障害非発現量(900 μg/kg/日[52])にもとづいて上限量を策定している．そこで

2010 年版では，ラットの健康障害非発現量にヨーロッパ食品科学委員会[51]が用いている不確実性因子 100 を適用し，9 μg/kg/ 日（体重 60 kg のヒトで 540 μg/ 日）をモリブデンの耐容上限量の基準値とした．米国における出納実験[53]からは，ヒトにおけるモリブデンの健康障害非発現量を 18 μg/kg/ 日と解釈できるため，上記の 9 μg/kg/ 日はヒトの健康障害非発現量に不確実性因子 2 を適用したことにもなる．

なお，乳児の目安量に関して，クロムと同様に日本人の母乳中濃度に関する報告[46)54)]が新たに提出されたため，2010 年版において初めてこれを策定した．

おわりに

以上，微量ミネラルの食事摂取基準策定の根拠と今後の課題について，成人の平均推定必要量と耐容上限量を中心に解説した．いずれのミネラルに関しても，2005 年版に比較して策定根拠はより明快になったものと判断している．2015 年版に向けては，本文でふれたことに加えて，離乳食からの微量ミネラル摂取量，サプリメントやミネラル強化食品摂取による大量摂取者の把握なども必要と思われる．食事摂取基準策定に必要な情報を得るための研究は地味であり，脚光を浴びることは少ない．しかし，栄養学が定量的な学問であり，その目的が人々に「何を」「どれだけ」「どのようにして」食べるのがよいかを示すことにあると信じている立場からは，より多くの栄養学研究者が食事摂取基準に注目し，その策定に活用できる研究に取り組まれることを期待している．

文 献

1) 厚生労働省 (2009) 日本人の食事摂取基準 [2010 年版]，pp. 218-267，第一出版，東京
2) 吉田宗弘 (2009) 微量ミネラル．臨床栄養 **115**，553-564.
3) Food and Nutrition Board, Institute of Medicine (2001) "Iron" in 'Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc' pp. 290-393, National Academy Press. Washington D.C.
4) Asakura K, Sasaki S, Murakami K, Takahashi Y, Uenishi K, Yamakawa M, Nishiwaki Y, Kikuchi Y, Takebayashi T (2009) Iron intake does not significantly correlate with iron deficiency among young Japanese women: a cross-sectional study. *Public Health Nutr* **12**, 1373-1383
5) Yokoi K (2003) Numerical methods for estimating iron requirements from population data. *Biol Trace Elem Res* **95**, 155-172
6) 矢野知佐子，富安俊子，穴井孝信 (2005) 正常月経周期日数とその変動範囲に関する調査．母性衛生 **45**，496-502
7) 野上保治 (1966) 経血量に関する研究．日本不妊学会雑誌 **11**，189-203.
8) FAO/WHO (1988) Requirements of vitamin A, iron, folate and vitamin B_{12} (FAO Food and Nutrition Series No. 23), pp. 33-50, FAO/WHO, Rome
9) Bothwell TH, Charlton RW (1981) Iron deficiency in women. pp. 7-9, The Nutrition Foundation, Washington D.C
10) Takimoto H, Yoshiike N, Katagiri A, Ishida H, Abe S (2003) Nutritional status of pregnant and lactating women in Japan: A comparison with non-pegnant/non-lactating controls in the National Nutrition Survey. *J Obstet Gynaecol Res* **29**, 96-103
11) Kamei A, Uenishi K, Ishida M, Suzuki H (2001) Iron intake and absorption in pregnant and lactating women. *Ann Nutr Metab* **45** (Suppl), 44-45.
12) FAO/WHO (1983) Evaluation of certain food additives and contaminants. Twenty-seventh report of the Joint FAO/WHO Committee on Food Additives (WHO Technical Report Series, No. 696). FAO/WHO, Rome
13) Food and Nutrition Board, Institute of Medicine (2001) "Zinc" in 'Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc' pp. 442-501, National Academies Press, Washington D.C
14) 鈴木和春，五島孜郎，菅家祐輔 (1983) 小児期における亜鉛および銅出納について，日本栄養・食糧学会誌 **36**，231-237
15) Fosmire G (1990) Zinc toxicity. *Am J Clin Nutr* **51**, 225-227
16) Turnlund JR, Keen CL, Smith RG (1990) Copper status and urinary and salivary copper in young men at three levels of dietary copper. *Am J Clin Nutr* **51**, 658-664
17) Turnlund JR, Scott KC, Peiffer GL, Jang AM, Keyes WR, Keen CL, Sakanashi TM (1997) Copper status of young men consuming a low-copper diet. *Am J Clin Nutr* **65**, 72-78
18) Food and Nutrition Board, Institute of Medicine (2001) "Copper" in 'Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc' pp. 224-257, National Academies Press, Washington D.C
19) Araya M, Olivares M, Pizarro F, González M, Speisky H, Uauy R (2003) Gastrointestinal symptoms and blood indicators of copper load in apparently healthy adults undergoing controlled copper exposure. *Am J Clin Nutr* **77**, 646-650.
20) 白石久二雄 (1994) 微量元素の摂取量．臨床栄養 **84**，381-389
21) Food and Nutrition Board, Institute of Medicine (2001) "Manganese" in 'Dietary reference intake for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc' pp. 394-419, National Academies Press, Washington D. C
22) Food and Nutrition Board, Institute of Medicine (2001) "Iodine" in 'Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc' pp. 258-289, National Academies Press, Washington D. C
23) Gardner DF, Centor RE, Utiger RD (1988) Effects of low dose oral iodine supplementation on thyroid function in normal men. *Clin Endocrinol* **28**, 283-288
24) Suzuki M, Tamura T (1985) Iodine intake of Japanese male university students: Urinary iodine excretion of sedentary and physically active students and sweat iodine excretion during exercise. *J Nutr Sci*

Vitaminol **31**, 409-415

25) Katamine S, Mamiya Y, Sekimoto K, Hoshino N, Totsuka K, Naruse U, Watabe A, Sugiyama R, Suzuki M (1986) Iodine content of various meals currently consumed by urban Japanese. *J Nutr Sci Vitaminol* **32**, 487-495

26) Konno N, Makita H, Yuri K, Iizuka N, Kawasaki K (1994) Association between dietary iodine intake and prevalence of subclinical hypothyroidism in the coastal regions of Japan. *J Clin Endocrinol Metab* **78**, 393-397

27) Zhao J, Wang P, Shang L, Sullivan KM, van der Haar F, Maberly G (2000) Endemic goiter associated with high iodine intake. *Am J Public Health* **90**, 1633-1635

28) Seal AJ, Creeke PI, Gnat D, Abdalla F, Mirghani Z (2006) Excess dietary iodine intake in long-term African refugees. *Public Health Nutr* **9**, 35-39

29) Fuse Y, Saito N, Tsuchiya T, Shishiba Y, Irie M (2007) Smaller thyroid gland volume with high urinary iodine excretion in Japanese schoolchildren: Normative reference values in an iodine-sufficient area and comparison with the WHO/ICCIDD reference. *Thyroid* **17**, 145-155

30) Nagataki S (2008) The average of dietary iodine intake due to the ingestion of seaweed is 1.2 mg/day in Japan. *Thyroid* **18**, 667-668

31) 今野則道, 飯塚徳男, 川崎君王, 田口英雄, 三浦錦一, 田口静子, 村上茂樹, 萩原康司, 野田靖子, 鵜川純男(1994)北海道在住成人における甲状腺疾患の疫学的調査－ヨード摂取量と甲状腺機能との関係－. 北海道医誌 **69**, 614-626

32) Zimmermann MB, Ito Y, Hess SY, Fujieda K, Molinari L (2005) High thyroid volume in children with excess dietary iodine intake. *Am J Clin Nutr* **81**, 840-844

33) Nishiyama S, Mikeda T, Okada T, Nakamura K, Kotani T, Hishinuma A (2004) Transient hypothyroidism or persistent hyperthyrotropinemia in neonates born to mothers with excessive iodine intake. *Thyroid* **14**, 1077-1083

34) WHO/FAO/IAEA (1996) "Selenium" in 'Trace elements in human nutrition and health' pp. 105-122, WHO, Geneva

35) Food and Nutrition Board, Institute of Medicine (2001) "Selenium" in 'Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids' pp. 284-324, National Academy Press, Washington D. C

36) Yang G-Q, Zhou R-H (1994) Further observations on the human maximum safe dietary selenium intake in a seleniferous area of China. *J Trace Elem Electrolytes Health Dis* **8**, 159-165

37) Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, Combs GF, Cappuccio FP, Ceriello A, Reid ME (2007) Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. *Ann Intern Med* **147**, 217-223

38) Zhuo H, Smith AH, Steinmaus C (2004) Selenium and lung cancer: A quantitative analysis of heterogeneity in the current epidemiological literature. *Cancer Epidemiol Biomarkers Prev* **13**, 771-778

39) Knekt P, Aromaa A, Maatela J, Alfthan G, Aaran RK, Hakama M, Hakulinen T, Peto R, Teppo L (1990) Serum selenium and subsequent risk of cancer among Finnish men and women. *J Natl Cancer Inst* **82**, 864-868

40) Kabuto M, Imai H, Yonezawa C, Neriishi K, Akiba S, Kato H, Suzuki T, Land CE, Blot WJ(1994) Prediagnostic serum selenium and zinc levels and subsequent risk of lung and stomach cancer in Japan. *Cancer Epidemiol Biomarkers Prev* **3**, 465-469

41) Food and Nutrition Board, Institute of Medicine (2001) "Chromium" in 'Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc' pp. 197-223, National Academy Press, Washington D. C.

42) Bunker VW, Lawson MS, Delves HT, Clayton BE (1984) The uptake and excretion of chromium by the elderly. *Am J Clin Nutr* **39**, 797-802

43) Offenbacher EG, Spencer H, Dowling HJ, Pi-Sunyer FX (1986) Metabolic chromium balances in men. *Am J Clin Nutr* **44**, 77-82

44) Balk EM, Lau J, Tatsioni A, Pittas AG, Lichtenstein AH (2007) Effect of chromium supplementation on glucose metabolism and lipids: A systematic review of randomized controlled trials. *Diabetes Care* **30**, 2134-2163

45) Lamson DW, Plaza SM (2002) The safety and efficacy of high-dose chromium. *Altern Med Rev* **7**, 218-235

46) Yoshida M, Takada A, Hirose J, Endô M, Fukuwatari T, Shibata K (2008) Molybdenum and chromium concentrations in breast milk from Japanese women. *Biosci Biotechnol Biochem* **72**, 2247-2250

47) Kovalsky VV, Yarovaya GA, Shmavonyan DM (1961) The change in purine metabolism of humans and animals under the conditions of molybdenum biogeochemical provinces. *Zh Obshch Biol* **22**, 179-191

48) Hattori H, Ashida A, Itô C, Yoshida M (2004) Determination of molybdenum in foods and human milk, and an estimation of average molybdenum intake in the Japanese population. *J Nutr Sci Vitaminol* **50**, 404-409

49) Yoshida M, Hattori H, Ôta S, Yoshihara K, Kodama N, Yoshitake Y, Nishimuta M (2006) Molybdenum balance in healthy young Japanese women. *J Trace Elem Med Biol* **20**, 245-252

50) Food and Nutrition Board, Institute of Medicine (2001) "Molybdenum" in 'Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc' pp. 420-441, National Academy Press, Washington D. C

51) Scientific Committee on Food (2000) Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Molybdenum. European Commission, Brussels, download from http://ec.europa.eu/food/fs/sc/scf/out80h_en.pdf

52) Fungwe TV, Buddingh F, Demick DS, Lox CD, Yang MT, Yang SP (1990) The role of dietary molybdenum on estrous activity, fertility, reproduction and molybdenum and copper enzyme activities of female rats. *Nutr Res* **10**, 515-524

53) Turnlund JR, Keyes WR, Peiffer GL (1995) Molybdenum absorption, excretion, and retention studied with stable isotopes in young men at five intakes of dietary molybdenum. *Am J Clin Nutr* **62**, 790-796

54) 吉田宗弘, 伊藤智恵, 服部浩之, 芦田薗, 米久保明得, 西牟田守(2004)日本における母乳および調整粉乳中のモリブデン濃度と乳児のモリブデン摂取量. 微量栄養素研究 **21**, 59-64.

【学会発表　要旨】

第 12 回日本骨粗鬆症学会（大阪）　2010 年 10 月 23 日

ダブルアイソトープ法による閉経後女性のカルシウム吸収率の検討

上西一弘、藤田拓男、石田裕美、藤井芳夫、大植　睦、梶博史、平井みどり、角本幹夫、Abrams SA

【目的】カルシウムは腸管からの吸収率が低い栄養素の 1 つであり、その吸収率は成人では 25%程度とされている。また、吸収率は個人差が大きく、加齢により減少することも知られている。しかし、個人ごとの腸管からのカルシウム吸収率を測定することは非常に困難であり、ほとんど行われてはいない。カルシウムの安定同位体（アイソトープ）を用いて吸収率を測定する方法がある。経口摂取するカルシウムの一部をアイソトープでラベルして尿中への排泄量を調べるシングルアイソトープ法はこれまでにわが国でも実施されているが、経口摂取と同時に、静脈中にもう 1 種類の異なるアイソトープを投与し、2 種類のアイソトープを用いて吸収率を測定する方法、ダブルアイソトープ法は、海外では実施されているが日本での実施例はない。今回我々はダブルアイソトープ法を用いて閉経後女性のカルシウム吸収率を測定することを目的に本研究を行った。

【方法】対象者は平均年齢 67±5 歳の閉経後女性 9 名であり、閉経後年数の平均は 17 年であった。44Ca を経口投与、42Ca を静脈投与した。経口投与したカルシウムは炭酸カルシウムの形態で、300mg 中 30mg が 44Ca である。静脈中には 3mg の 42Ca を投与した。24 時間の採尿を行い、尿中の 44Ca 、42Ca を ICP-MS で測定し、腸管からのカルシウム吸収率を計算した。本研究は香川栄養学園、神戸大学、藤井クリニックの各倫理委員会の承認を得て実施した。

【結果】対象者のカルシウム栄養状態、骨代謝マーカーでは 1 名の血清 25（OH）ビタミン D 濃度が 7.6ng/dl と低値であった以外は、特に問題はなかった。ダブルアイソトープ法によるカルシウムの吸収率は 14.7±6.4%であり、6.1%から 22.2%の範囲にあった。

【結語】ダブルアイソトープ法により測定した閉経後女性のカルシウムの吸収率は 14.7±6.4%であり、個人差も大きかった。本法は比較的簡便にカルシウムの吸収率を測定することができ、基礎研究及び臨床研究に役立つものと期待される。