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Circulating Levels of Fatty Acid-Binding Protein Family and Metabolic Phenotype in the General Population

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Abstract

Objective: Fatty acid-binding proteins (FABPs) are a family of 14-15-kDa proteins, and some FABPs have been to be used as biomarkers of tissue injury by leak from cells. However, recent studies have shown that FABPs can be secreted from cells into circulation. Here we examined determinants and roles of circulating FABPs in a general population.

Methods: From the database of the Tanno-Sobetsu Study, a study with a population-based cohort design, data in 2011 for 296 subjects on no medication were retrieved, and FABP1~5 in their serum samples were assayed.

Results: Level of FABP4, but not the other isoforms, showed a gender difference, being higher in females than in males. Levels of all FABPs were negatively correlated with estimated glomerular filtration rate (eGFR), but a distinct pattern of correlation with other clinical parameters was observed for each FABP isoform; significant correlates were alanine aminotransferase (ALT), blood pressure (BP), and brain natriuretic peptide (BNP) for FABP1, none besides eGFR for FABP2, age, BP, and BNP for FABP3, age, waist circumference (WC), BP, BNP, lipid variables, high-sensitivity C-reactive protein (hsCRP), and HOMA-R for FABP4, and age, WC, BP, ALT, BNP, and HOMA-R for FABP5. FABP4 is the most strongly related to metabolic markers among FABPs. In a multivariate regression analysis, FABP4 level was an independent predictor of HOMA-R after adjustment of age, gender, WC, BP, HDL cholesterol, and hsCRP.

Conclusions: Each FABP isoform level showed a distinct pattern of correlation with clinical parameters, although levels of all FABPs were negatively determined by renal function. Circulating FABP4 appears to be a useful biomarker for detecting pre-clinical stage of metabolic syndrome, especially insulin resistance, in the general population.

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Introduction

Intracellular lipid chaperones known as fatty acid-binding proteins (FABPs) are a group of molecules that coordinate lipid responses in cells. FABPs are abundantly expressed 14-15-kDa proteins that can reversibly bind hydrophobic ligands such as saturated and unsaturated long chain fatty acids with high affinity [1,2]. FABPs have been proposed to facilitate the transport of lipids to specific compartments in the cell. At least nine distinct types of FABP have been identified, and each type has a characteristic pattern of tissue distribution. The FABP types are named after the tissues in which they were first identified, and the FABP family consists of liver-type (FABP1/L-FABP), intestinal-type (FABP2/I-FABP), heart-type (FABP3/H-FABP), adipocyte-type (FABP4/A-FABP), epidermal-type (FABP5/E-FABP), ileal-type (FABP6/Il-FABP), brain-type (FABP7/B-FABP), myelin-type (FABP8/M-FABP), and testis-type FABPs (FABP9/T-FABP) [1]. However, the tissue/cell-type classification of FABPs is somewhat misleading, since no FABP is exclusively specific to a given tissue or cell type, and most tissues express several FABP isoforms [1]: e.g., FABP1 in the kidney and intestine, FABP2 in the liver, FABP3 in liver, FABP4 in macrophages, and FABP5 in adipocytes, macrophages, liver, and heart.

Numerous studies have recently shown the presence of FABPs in circulation. Since FABPs lack a secretory signal sequence, the presence of FABPs in serum has been considered to be a promising tissue-specific marker of tissue injury: FABP1 for liver damage [3], FABP2 for intestinal injury [4,5], and FABP3 for acute myocardial infarction and ongoing myocardial damage in heart failure [6,7].

However, it has recently been reported that FABP4 is secreted from adipocytes [8]. Furthermore, increased serum concentration of FABP4 has been shown to be associated with obesity, type 2 diabetes, hypertension, and cardiovascular diseases [8–11]. Similar findings have also been reported for FABP5 [12,13]. However, the significance of serum concentrations of FABPs in the general population has not been elucidated. In the present study, we determined serum concentrations of FABP1, FABP2, FABP3, FABP4, and FABP5 in Japanese subjects on no medication and investigated the relationships of the concentration of each FABP isoform with tissue damage and metabolic phenotype.

Methods

Study population

In the Tanno-Sobetsu Study, a study with a population-based cohort design, a total of 617 Japanese subjects (male/female: 260/357, mean age: 65.8±0.5 years) were recruited from residents of two rural towns, Tanno and Sobetsu, in Hokkaido, the northernmost island of Japan, in 2011. Subjects who were being treated with any medications were excluded, and subjects who were not on any medication (n = 296, male/female: 122/174) were enrolled in the present analyses. This study conformed to the principles outlined in the Declaration of Helsinki and was performed with the approval of the institutional ethical committee of Sapporo Medical University. Written informed consent was received from all of the subjects.

Measurements

Medical check-ups were performed between 06:00 h and 09:00 h after an overnight fast. After measuring anthropometric parameters, blood pressure was measured twice consecutively on the upper arm using an automated sphygmomanometer (HEM-907, Omron Co., Kyoto, Japan) with subjects in a seated resting position, and average blood pressure was used for analysis. Body mass index (BMI) was calculated as body weight (in kilograms) divided by the square of body height (in meters).

Peripheral venous blood samples were obtained from study subjects after physical examination for complete blood count and biochemical analyses of the serum. The serum samples were analyzed immediately or stored at −80°C until biochemical analyses. Concentrations of FABPs in serum samples were measured using commercially available enzyme-linked immunosorbent assay kits for FABP1 (CIMIC Co., Tokyo, Japan), FABP2 (Hycult Biotech, Uden, Netherlands), FABP3 (DS Pharma Biomedical Co., Osaka, Japan), FABP4 (Biovendor R&D, Modrice, Czech Republic), and FABP5 (USCN Life Science, Houston, U.S.A.). The accuracy, precision and reproducibility of the kits for FABP1, FABP2, FABP3, and FABP4 have been described previously [8,14–16]. The intra- and inter-assay coefficient variances in the kits were <15%. According to manufacturer's protocol, no cross-reactivity of FABP5 with other FABP types was observed. Fasting plasma glucose was determined by the glucose oxidase method. Fasting plasma insulin was measured by a radioimmunoassay method (Insulin RIA bead, Dianabot, Tokyo, Japan). Creatinine (Cr), aspartate transaminase (AST), alanine aminotransferase (ALT), and lipid profiles, including total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides, were determined by enzymatic methods. Low-density lipoprotein (LDL) cholesterol level was calculated by the Friedewald equation. Brain natriuretic peptide (BNP) was measured using an assay kit (Shionogi & Co., Osaka, Japan). High-sensitivity C-reactive protein (hsCRP) was measured by a nephelometry method.

As an index of renal function, estimated GFR (eGFR) was calculated by an equation for Japanese [17]: eGFR (mL/min/1.73 m^2) = $194 \times Cr^{(-1.094)} \times age^{(-0.287)} \times 0.739$ (if female). HOMA-R, an indicator of insulin resistance, was calculated by the previously reported formula: insulin (μU/ml)×glucose (mg/dl)/405.

Statistical analysis

Numeric variables are expressed as means ± SEM. The distribution of each parameter was tested for its normality using the Shapiro-Wilk W test, and non-normally distributed parameters were logarithmically transformed. Comparison between two groups was done with an unpaired *t* test. The correlation between two variables was evaluated using Pearson's correlation coefficient. Multiple linear regression analysis was performed to identify independent determinants of each FABP concentration and HOMA-R using the variables with a significant and non-confounding correlation in simple regression analysis as independent predictors, showing the t-ratio calculated as the ratio of regression coefficient and standard error of regression coefficient and the percentage of variance in the each FABP concentration or HOMA-R that they explained (R^2). A p value of less than 0.05 was considered statistically significant. All data were analyzed by using JMP 9 for Macintosh (SAS Institute, Cary, NC).

Results

Serum levels of FABPs

Demographic characteristics of the 296 recruited subjects (male/female: 122/174) are shown in Table 1. There was no significant difference in age, systolic blood pressure, eGFR, and BNP level between the male and female subjects. Total, HDL, and LDL cholesterol levels were significantly higher in females than in males. Indices of adiposity (BMI and waist circumference), indices of glucose metabolism (glucose, insulin, and HOMA-R), and Cr, AST, ALT, and hsCRP were higher in males than in females. There were approximately 30-fold differences in serum levels of FABPs depending on the isoform: FABP1 (male, female: 3.4±0.1, 3.3±0.1 ng/ml), FABP2 (male, female: 0.30±0.01, 0.27±0.01 ng/ml), FABP3 (male, female: 3.5±0.4, 3.9±0.4 ng/ml), FABP4 (male, female: 10.0±0.6, 13.3±0.5 ng/ml), and FABP5 (male, female: 1.8±0.1, 1.7±0.1 ng/ml) (Figure 1A). FABP4 concentration was the highest among levels of FABPs and was significantly higher in females than in males. No other concentrations of FABPs showed a significant gender difference.

Correlations between FABPs levels and clinical parameters

Table 2 shows a summary of the results of univariate regression analyses for each FABP isoform. Serum levels of all of the FABPs were negatively correlated with eGFR. FABP1 level was positively correlated with age, systolic blood pressure, triglycerides, AST, ALT, and BNP. None of the parameters determined in this study, except for eGFR, was significantly correlated with FABP2 concentration, although there were trends for FABP2 to correlate with triglycerides (r = 0.11, p = 0.058) and to negatively correlate with HDL cholesterol (r = −0.11, p = 0.050). FABP3 level was positively correlated with age, systolic and diastolic blood pressures, and BNP. FABP4 level was negatively correlated with HDL cholesterol and was positively correlated with age, BMI, waist circumference, systolic and diastolic blood pressures, total and LDL cholesterol, triglycerides, glucose, insulin, HOMA-R, BNP, and hsCRP. FABP5 level correlated positively with age,

Table 1. Characteristics of the studied subjects.

	Total	Male	Female
n	296	122	174
Age (years)	60.4±0.9	60.8±1.4	60.1±1.1
Body mass index (kg/m^2)	22.7±0.2	23.5±0.3	22.1±0.2**
Waist circumference (cm)	82.9±0.6	85.8±0.9	80.8±0.7**
Systolic blood pressure (mmHg)	133.2±1.4	135.1±2.0	131.8±1.9
Diastolic blood pressure (mmHg)	76.5±0.7	78.4±1.2	75.2±0.9*
Biochemical data			
Total cholesterol (mg/dl)	203.3±2.0	195.0±3.1	209.0±2.5**
HDL cholesterol (mg/dl)	67.9±1.1	59.5±1.6	73.8±1.3**
LDL cholesterol (mg/dl)	121.9±1.7	117.8±2.8	124.7±2.2*
Triglycerides (mg/dl)	109.0±6.1	145.9±5.7	84.9±2.9**
Glucose (mg/dl)	97.7±3.0	97.9±1.6	92.3±1.0**
HbA1c (%)	5.03±0.03	5.11±0.04	4.97±0.03*
Insulin (μU/ml)	5.8±0.3	6.7±0.5	5.2±0.2**
HOMA-R	1.41±0.08	1.69±0.17	1.22±0.07**
Cr (mg/dl)	0.74±0.01	0.84±0.01	0.66±0.01**
estimated GFR (ml/min/1.73 m^2)	73.8±0.8	75.2±1.3	72.8±1.1
AST (IU/l)	23.6±0.5	26.1±0.9	21.8±0.5**
ALT (IU/l)	21.5±0.7	26.3±1.3	18.1±0.7**
BNP (pg/ml)	26.2±1.9	24.1±3.7	27.7±1.9
hsCRP (mg/dl)	0.064±0.005	0.083±0.009	0.052±0.005**

Variables are expressed as n or means ± SEM.
*P<0.05,
**P<0.01 vs. male.
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waist circumference, systolic blood pressure, glucose, HOMA-R, AST, ALT, and BNP.

Results of multiple regression analyses for each FABP isoform are shown in Table 3. As independent determinants, ALT for FABP1, eGFR for FABP2 and FABP3, age, gender, waist circumference, and eGFR for FABP4, and age and eGFR for FABP5 were selected (Table 3).

FABP4 level as a metabolic biomarker in the general population

Results of univariate and multivariate regression analyses (Tables 2 and 3) indicated that FABP4 is the most strongly related to metabolic markers among FABPs. Hence, we next focused on the significance of FABP4 in metabolic disorders, especially insulin resistance, in the study subjects. Serum FABP4 level was positively correlated with HOMA-R ($r=0.32$, $p<0.001$) as shown in Figure 1B. When the subjects were divided into male and female subjects, there was a still significant correlation between FABP4 level and HOMA-R in each gender (male: $r=0.40$, $p<0.001$; female: $r=0.38$, $p<0.001$) (Table 4). HOMA-R was also negatively correlated with HDL cholesterol and was positively correlated with age, BMI, waist circumference, systolic and diastolic blood pressures, LDL cholesterol, triglycerides, and hsCRP (Table 4). Multiple regression analysis using age, gender, and the non-confounding correlated parameters revealed that serum FABP4 concentration was an independent predictor of HOMA-R, explaining a total of 40.6% of the variance in this measure ($R^2=0.406$) (Table 5).

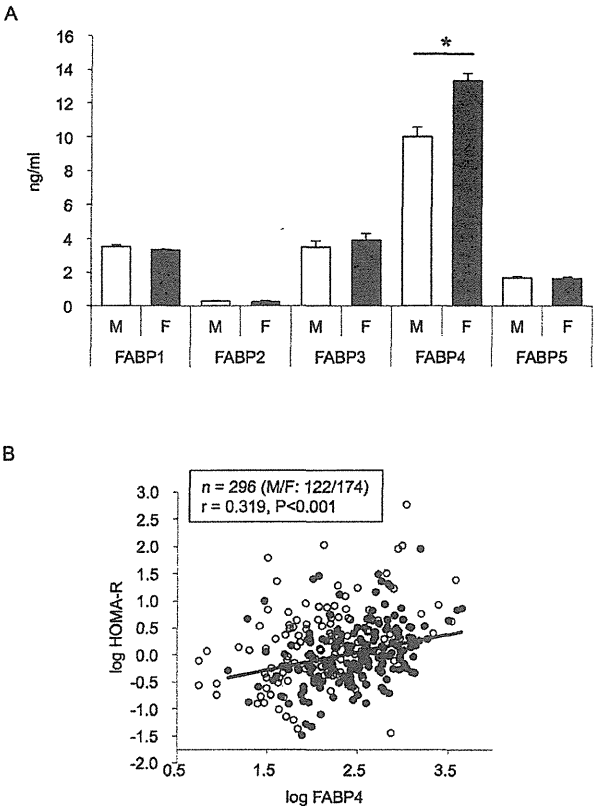

Figure 1. Concentrations of FABPs. A. Bar graphs show serum concentrations of FABP1, FABP2, FABP3, FABP4, and FABP5 in male (n=122) and female (n=174) subjects on no medication. Values are presented as means ± SEM. *P<0.001. B. Log HOMA-R was plotted against log FABP4 in each study subject. There was a significant correlation between the two parameters (n=296, r=0.319, P<0.001). Open circle: male (n=122), closed circle: female (n=174).
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Discussion

To the best of our knowledge, this is the first report on the circulating level of each FABP in a general population on no medications. Although none of the FABPs are tissue-specific, the FABP family has drawn interest recently as early and sensitive serum markers of tissue damage or injury. The present study showed that concentrations of FABPs except for FABP2 were correlated with distinct biochemical markers reflecting tissue damage in subjects on no medication: i.e., FABP1 for liver damage, FABP3 for cardiac injury, FABP4 for adiposity and metabolic syndrome, and FABP5 for adiposity, insulin resistance, cardiac injury, and liver damage. FABP2 was not correlated with any tissue injury markers, presumably because none of the clinical parameters determined in this study are specific and sensitive markers of intestinal injury. In addition, we found that serum concentrations of FABP1~FABP5 negatively correlated with eGFR. The results are consistent with results of previous studies showing that levels of several FABPs were increased in subjects with renal dysfunction [3,18–20] and indicate that FABPs are eliminated from the circulation mainly by renal clearance. Hence, though eGFR needs to be taken into account in interpretation of serum FABP levels, relatively high tissue concentrations of FABPs and their low serum concentrations under normal conditions

Table 2. Simple regression analysis for log FABPs.

	log FABP1		log FABP2		log FABP3		log FABP4		log FABP5	
	r	p	r	p	r	p	r	p	r	p
Age	0.201	0.001*	0.073	0.210	0.232	<0.001*	0.309	<0.001*	0.348	<0.001*
Body mass index	0.107	0.068	−0.009	0.872	−0.002	0.975	0.454	<0.001*	0.110	0.059
Waist circumference	0.103	0.078	−0.004	0.949	0.015	0.804	0.458	<0.001*	0.141	0.015*
Systolic blood pressure	0.154	0.008*	0.022	0.712	0.187	0.001*	0.257	<0.001*	0.193	<0.001*
Diastolic blood pressure	0.046	0.430	−0.001	0.998	0.137	0.018*	0.214	<0.001*	0.067	0.251
Total cholesterol	0.092	0.118	0.026	0.661	−0.003	0.957	0.255	<0.001*	0.037	0.532
LDL cholesterol	0.077	0.186	0.066	0.262	0.059	0.315	0.257	<0.001*	0.013	0.823
HDL cholesterol	0.011	0.848	−0.114	0.050	0.043	0.461	−0.075	0.196	−0.042	0.467
log Triglycerides	0.125	0.032*	0.110	0.058	−0.112	0.054	0.187	0.001*	0.085	0.145
Glucose	0.074	0.208	0.033	0.578	−0.008	0.889	0.154	0.008*	0.175	0.003*
log Insulin	−0.011	0.855	−0.002	0.977	0.031	0.600	0.311	<0.001*	0.112	0.054
log HOMA-R	0.006	0.924	0.005	0.927	0.034	0.562	0.319	<0.001*	0.143	0.014*
eGFR	−0.119	0.042*	−0.160	0.006*	−0.229	<0.001*	−0.386	<0.001*	−0.331	<0.001*
log AST	0.232	<0.001*	0.088	0.132	0.104	0.075	0.112	0.055	0.186	0.001*
log ALT	0.226	<0.001*	0.059	0.313	0.061	0.299	0.016	0.781	0.134	0.022*
log BNP	0.156	0.007*	0.065	0.269	0.211	<0.001*	0.176	0.002*	0.175	0.003*
log hsCRP	0.067	0.260	−0.021	0.722	0.083	0.163	0.183	0.002*	0.108	0.068

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would enable serum FABP levels to be novel and sensitive biomarkers.

FABP1

No influence of gender was observed for FABP1 in serum, being consistent with a previous report [3]. FABP1 level was correlated with AST and ALT (Table 2), potentially reflecting liver injury. Serum AST, ALT and lactate dehydrogenease are commonly used as plasma markers of acute hepatocellular injury for detection and monitoring of liver disease. Although ALT is a well-established specific, quickly measurable and cost-effective biomarker of hepatocellular injury, it is a relatively large protein (96 kDa) and slowly indicates cell damage. A study recruiting liver transplant recipients showed that elevation of serum FABP1 level after hepatocellular injury due to rejection was larger and faster than that of ALT, indicating that FABP1 is a more sensitive marker [3]. In the present study, we recruited only apparently healthy subjects on no medication and confirmed that their serum biochemical parameters, including AST and ALT, were within normal ranges. Thus, correlation between serum FABP1 concentration and "normal" levels of AST and ALT indicates that serum FABP1 is very sensitive marker of liver injury or injurious stress on the liver.

Regarding metabolic phenotype, it has been reported that FABP1-deficient mice were of normal weight and that serum levels

Table 3. Multiple regression analysis for log FABPs.

	FABP1		FABP2		FABP3		FABP4		FABP5	
	t	p	t	p	t	p	t	p	t	p
Age	1.27	0.205	−0.37	0.711	0.51	0.609	2.11	0.035*	3.02	0.003*
Gender (Male)	0.01	0.995	1.41	0.159	0.87	0.383	−8.69	<0.001*	0.09	0.930
Waist circumference	-	-	-	-	-	-	8.59	<0.001*	0.37	0.711
Systolic blood pressure	0.38	0.706	-	-	1.14	0.256	−0.09	0.931	−0.53	0.598
Total cholesterol	-	-	-	-	-	-	1.76	0.08	-	-
log Triglycerides	0.64	0.522	-	-	-	-	-	-	-	-
log HOMA-R	-	-	-	-	-	-	1.33	0.185	1.23	0.220
eGFR	−0.4	0.687	−2.63	0.009*	−2.12	0.035*	−5.07	<0.001*	−3.16	0.002*
log ALT	3.54	0.001*	-	-	-	-	-	-	1.90	0.059
log BNP	1.35	0.178	-	-	1.8	0.073	−1.4	0.162	−0.01	0.996
log hsCRP	-	-	-	-	-	-	0.56	0.573	-	-
	$R^2 = 0.10$		$R^2 = 0.03$		$R^2 = 0.09$		$R^2 = 0.51$		$R^2 = 0.18$	

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Table 4. Simple regression analysis for log HOMA-R.

	Total		Male		Female	
	r	p	r	p	r	p
Age	0.030	0.609	−0.030	0.744	0.080	0.292
Body mass index	0.558	<0.001*	0.602	<0.001*	0.491	<0.001*
Waist circumference	0.545	<0.001*	0.569	<0.001*	0.500	<0.001*
Systolic blood pressure	0.234	<0.001*	0.160	0.079	0.287	<0.001*
Diastolic blood pressure	0.215	<0.001*	0.121	0.185	0.276	<0.001*
Total cholesterol	0.018	0.760	0.159	0.081	−0.048	0.534
LDL cholesterol	0.127	0.029*	0.224	0.014*	0.076	0.319
HDL cholesterol	−0.394	<0.001*	−0.389	<0.001*	−0.348	<0.001*
log Triglycerides	0.376	<0.001*	0.410	<0.001*	0.289	<0.001*
log hsCRP	0.227	<0.001*	0.236	0.010*	0.177	0.022*
log FABP4	0.319	<0.001*	0.403	<0.001*	0.378	<0.001*

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Table 5. Multiple regression analysis for log HOMA-R.

	t	p
Age	−3.32	0.001*
Gender (Male)	−0.23	0.821
Waist circumference	6.49	<0.001*
Systolic blood pressure	3.3	0.001*
HDL cholesterol	−4.42	<0.001*
log hsCRP	0.62	0.539
log FABP4	2.03	0.044*
	$R^2 = 0.41$	

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of triglycerides and fatty acids were unchanged [21,22]. Changes in metabolic parameters caused by a high fat/cholesterol diet in FABP1-deficient mice differed between studies [23,24]. It is possible that the principal action of FABP1 is not serum lipid regulation but another action. Interestingly, recent studies have shown that urinary FABP1 in humans would be a useful clinical marker for prediction and monitoring of the progression of renal diseases [14,25]. In the present study, serum FABP1 concentration was correlated with systolic blood pressure and BNP, indicating a possible role in cardiovascular regulation. Since subjects with heart failure were generally excluded from the study subjects, it is unlikely that correlation of FABP1 and BNP is attributable to liver congestion by latent right ventricular failure.

FABP2

A polymorphism in FABP2, an alanine-to-threonine substitution at codon 54 (Thr-54), has been reported to be associated with insulin resistance in Pima Indians, a population with an extremely high prevalence of obesity and type 2 diabetes [26]. However, the association between the Thr-54 allele and insulin resistance in other populations has been controversial [27,28]. Previous studies showed the applicability of FABP2 for detection of intestinal injury after acute ischemic diseases, rejection and necrotic enterocolitis [4,5], whereas FABP2 concentrations in plasma of healthy individuals were reported to be undetectable or very low [5,29]. In the present study, we could detect FABP2 in serum of healthy subjects, and its level was lowest among FABPs. In contrast to the other FABP isoforms determined in this study, FABP2 level was not correlated with parameters relevant to glucose and lipid metabolism, blood pressure or BNP. These features of FABP2 appear to be advantageous as a biomarker of intestinal injury.

FABP3

FABP3 is abundant in the myocardium and is rapidly released from cells into the circulation after onset of cardiomyocyte damage. Serum concentration of FABP3 has been characterized as an early biochemical marker of acute myocardial infarction and a sensitive marker of myocardial damage in patients with heart failure [6,7]. In the present study, serum FABP3 level positively correlated with blood pressure, BNP and eGFR (Table 2), although only eGFR was selected as a significant determinant by multivariate analysis (Table 3). These results suggest that the association of FABP3 with blood pressure and BNP was mediated by decline of eGFR. However, recent studies have shown diastolic ventricular dysfunction in approximately 20% of asymptomatic subjects in the general population. Thus, it is possible that elevation of FABP3 after adjustment with eGFR indicates such asymptomatic ventricular dysfunction.

There was no significant gender difference in FABP3 level in subjects on no medication (Figure 1A). In contrast, higher serum FABP3 levels in males than in females were reported earlier in Japanese and Caucasian subjects, and the gender difference was attributed to larger muscle mass in males [30–32]. However, the study using Japanese subjects in the earlier studies [30,31] included those with hypertension, diabetes mellitus, dyslipidemia, and smoking habits, and incidences of coronary risk factors were higher in males than in females [30,31]. Thus, the reported gender difference in serum FABP3 level in Japanese subjects is likely to be an apparent one due to different myocardial insults between male and female groups. On the other hand, the other earlier study enrolled Caucasian healthy subjects and young volunteers [32]. It is also possible that there is a difference in muscle tissue distribution between Japanese and Caucasian subjects.

FABP4

Previous studies using animal models indicated that FABP4 plays a significant role in several aspects of metabolic syndrome, including insulin resistance, type 2 diabetes, and atherosclerosis, through its action at the interface of metabolic and inflammatory pathways in adipocytes and macrophages [33–36]. It has also been demonstrated that chemical inhibition of FABP4 could be a therapeutic strategy against insulin resistance, diabetes mellitus, fatty liver disease, and atherosclerosis in experimental models [37]. Interestingly, it has recently been shown that FABP4 is secreted from adipocytes, though there is no typical sequence of secretory signal peptides [8]. We previously confirmed that FABP4 release from adipocytes was not an escape due to apoptosis or necrosis of adipocytes [36], although the precise mechanisms underlying secretion of FABPs are still unclear. In this study, serum concentration of FABP4 was higher in females than in males, being consistent with results of previous studies [8–11,20]. This may be due to the larger amount of body fat in females than in males. Recent clinical studies have shown that elevation of serum FABP4 is associated with obesity, insulin resistance, hypertension, and atherosclerosis [8–11]. We and others have also shown that serum FABP4 level predicts long-term cardiovascular outcomes [20,38].

In the present study, we found that FABP4 level is associated with clinical parameters of obesity, insulin resistance, dyslipidemia, and high blood pressure even in asymptomatic apparently healthy

subjects with no pharmacological treatments. These findings raise the possibility that elevation of serum FABP4 is a very early event in the pathogenesis of insulin resistance and obesity. However, it is still unknown whether association of elevated circulating FABP4 level with insulin resistance is a result of direct physiological effects of FABP4 as a bioactive molecule *in vivo*. To address this issue, effects of recombinant FABP on metabolic phenotype need to be clearly demonstrated.

FABP5

FABP5 is expressed most abundantly in epidermal cells of the skin and is also present in other tissues, including adipocytes, macrophages, liver, and heart [1]. Ablation of FABP5 led to a mild increase in systemic insulin sensitivity in genetic and dietary obesity mouse models [39], whereas adipose tissue-specific overexpression of FABP5 in transgenic mice resulted in a reduction in systemic insulin sensitivity in a high-fat diet model [39]. In the present study, serum level of FABP5 was correlated with waist circumference and HOMA-R, indicating that FABP5 would be a metabolic marker, although the correlation was not as strong as that of FABP4.

In addition to metabolic parameters, AST and ALT levels were correlated with FABP5. Several lines of evidence indicate a role of FABP5 in the liver. Complete or partial lack of FABP5 in the liver during perinatal development was compensated by overexpression of FABP3 [40]. The expression of FABP5, but not that of FABP1, was increased in liver parenchymal cells by a western-type high-cholesterol diet in atherosclerotic LDL receptor-deficient mice [41]. These findings suggest that FABP5 has important and distinct roles in the liver, although there were no apparent morphological changes in the liver of FABP5-deficient mice [40]. Whether elevation of serum FABP5 and that of FABP1 indicate different types of liver injury is an interesting issue and remains to be investigated.

Study limitations

Our study has some limitations. First, this study is a cross-sectional design, which does not prove causal relations between serum levels of FABPs and the correlated biomarkers. A longitudinal study and interventional study are needed to clarify what underlies the relationship between FABPs and metabolic and tissue damage markers. Second, because the subjects of our study were only Japanese people, it is unclear whether the present findings can be generalized to other ethnicities. Lastly, it is not clear whether methods of each FABP isoform assay used in this study were suitable for diagnostic and prognostic use in terms of timely and definitive evaluation in clinical practice.

Conclusions

Serum levels of FAPB1~FABP5 showed distinct patterns of correlation with physiological and metabolic parameters in a general population without pharmacological treatments, although eGFR is a negative determinant of all FABP isoform levels. Of serum FABPs, FABP4 showed the closest correlations with metabolic parameters and was the only independent determinant of HOMA-R in a general population. Thus, this FABP isoform might be an early and useful biomarker of metabolic syndrome phenotype.

Author Contributions

Conceived and designed the experiments: MF. Performed the experiments: MF SI YW KH TF T. Mita YO MK MT. Analyzed the data: MF SI HA HO HY SS. Wrote the paper: MF SI T. Miura.

References

1. Furuhashi M, Hotamisligil GS (2008) Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. Nat Rev Drug Discov 7: 489–503.
2. Furuhashi M, Ishimura S, Ota H, Miura T (2011) Lipid chaperones and metabolic inflammation. Int J Inflam 2011: 642612.
3. Pelsers MM, Morovat A, Alexander GJ, Hermens WT, Trull AK, et al. (2002) Liver fatty acid-binding protein as a sensitive serum marker of acute hepatocellular damage in liver transplant recipients. Clin Chem 48: 2055–2057.
4. Pelsers MM, Hermens WT, Glatz JF (2005) Fatty acid-binding proteins as plasma markers of tissue injury. Clin Chim Acta 352: 15–35.
5. Pelsers MM, Namiot Z, Kisielewski W, Namiot A, Januszkiewicz M, et al. (2003) Intestinal-type and liver-type fatty acid-binding protein in the intestine. Tissue distribution and clinical utility. Clin Biochem 36: 529–535.
6. Tanaka T, Hirota Y, Sohmiya K, Nishimura S, Kawamura K (1991) Serum and urinary human heart fatty acid-binding protein in acute myocardial infarction. Clin Biochem 24: 195–201.
7. Setsuta K, Seino Y, Ogawa T, Arao M, Miyatake Y, et al. (2002) Use of cytosolic and myofibril markers in the detection of ongoing myocardial damage in patients with chronic heart failure. Am J Med 113: 717–722.
8. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, et al. (2006) Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. Clin Chem 52: 405–413.
9. Tso AW, Xu A, Sham PC, Wat NM, Wang Y, et al. (2007) Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. Diabetes Care 30: 2667–2672.
10. Yeung DC, Xu A, Cheung CW, Wat NM, Yau MH, et al. (2007) Serum adipocyte fatty acid-binding protein levels were independently associated with carotid atherosclerosis. Arterioscler Thromb Vasc Biol 27: 1796–1802.
11. Ota H, Furuhashi M, Ishimura S, Koyama M, Okazaki Y, et al. (2012) Elevation of fatty acid-binding protein 4 is predisposed by family history of hypertension and contributes to blood pressure elevation. Am J Hypertens 25: 1124–1130.
12. Yeung DC, Wang Y, Xu A, Cheung SC, Wat NM, et al. (2008) Epidermal fatty-acid-binding protein: a new circulating biomarker associated with cardio-metabolic risk factors and carotid atherosclerosis. Eur Heart J 29: 2156–2163.
13. Bagheri R, Qasim AN, Mehta NN, Terembula K, Kapoor S, et al. (2010) Relation of plasma fatty acid binding proteins 4 and 5 with the metabolic syndrome, inflammation and coronary calcium in patients with type-2 diabetes mellitus. Am J Cardiol 106: 1118–1123.
14. Kamijo A, Kimura K, Sugaya T, Yamanouchi M, Hikawa A, et al. (2004) Urinary fatty acid-binding protein as a new clinical marker of the progression of chronic renal disease. J Lab Clin Med 143: 23–30.
15. Morariu AM, Loef BG, Aarts LP, Rietman GW, Rakhorst G, et al. (2005) Dexamethasone: benefit and prejudice for patients undergoing on-pump coronary artery bypass grafting: a study on myocardial, pulmonary, renal, intestinal, and hepatic injury. Chest 128: 2677–2687.
16. Ohkaru Y, Asayama K, Ishii H, Nishimura S, Sunahara N, et al. (1995) Development of a sandwich enzyme-linked immunosorbent assay for the determination of human heart type fatty acid-binding protein in plasma and urine by using two different monoclonal antibodies specific for human heart fatty acid-binding protein. J Immunol Methods 178: 99–111.
17. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, et al. (2009) Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 53: 982–992.
18. Furuhashi M, Ura N, Hasegawa K, Yoshida H, Tsuchihashi K, et al. (2003) Serum ratio of heart-type fatty acid-binding protein to myoglobin. A novel marker of cardiac damage and volume overload in hemodialysis patients. Nephron Clin Pract 93: C69–74.
19. Furuhashi M, Ura N, Hasegawa K, Tsuchihashi K, Nakata T, et al. (2004) Utility of serum ratio of heart-type fatty acid-binding protein to myoglobin for cardiac damage regardless of renal dysfunction. Circ J 68: 656–659.
20. Furuhashi M, Ishimura S, Ota H, Hayashi M, Nishitani T, et al. (2011) Serum fatty acid-binding protein 4 is a predictor of cardiovascular events in end-stage renal disease. PLoS ONE 6: e27356.
21. Martin GG, Danneberg H, Kumar LS, Atshaves BP, Erol E, et al. (2003) Decreased liver fatty acid binding capacity and altered liver lipid distribution in mice lacking the liver fatty acid-binding protein gene. J Biol Chem 278: 21429–21438.
22. Newberry EP, Xie Y, Kennedy S, Han X, Buhman KK, et al. (2003) Decreased hepatic triglyceride accumulation and altered fatty acid uptake in mice with deletion of the liver fatty acid-binding protein gene. J Biol Chem 278: 51664–51672.
23. Martin GG, Atshaves BP, McIntosh AL, Mackie JT, Kier AB, et al. (2006) Liver fatty acid binding protein gene ablation potentiates hepatic cholesterol

accumulation in cholesterol-fed female mice. Am J Physiol Gastrointest Liver Physiol 290: G36–48.
24. Newberry EP, Xie Y, Kennedy SM, Luo J, Davidson NO (2006) Protection against Western diet-induced obesity and hepatic steatosis in liver fatty acid-binding protein knockout mice. Hepatology 44: 1191–1205.
25. Kamijo-Ikemori A, Sugaya T, Kimura K (2006) Urinary fatty acid binding protein in renal disease. Clin Chim Acta 374: 1–7.
26. Baier LJ, Sacchettini JC, Knowler WC, Eads J, Paolisso G, et al. (1995) An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation, and insulin resistance. J Clin Invest 95: 1281–1287.
27. Stan S, Lambert M, Delvin E, Paradis G, O'Loughlin J, et al. (2005) Intestinal fatty acid binding protein and microsomal triglyceride transfer protein polymorphisms in French-Canadian youth. J Lipid Res 46: 320–327.
28. Ito K, Nakatani K, Fujii M, Katsuki A, Tsuchihashi K, et al. (1999) Codon 54 polymorphism of the fatty acid binding protein gene and insulin resistance in the Japanese population. Diabet Med 16: 119–124.
29. Guthmann F, Borchers T, Wolfrum C, Wustrack T, Bartholomaus S, et al. (2002) Plasma concentration of intestinal- and liver-FABP in neonates suffering from necrotizing enterocolitis and in healthy preterm neonates. Mol Cell Biochem 239: 227–234.
30. Niizeki T, Takeishi Y, Takabatake N, Shibata Y, Konta T, et al. (2007) Circulating levels of heart-type fatty acid-binding protein in a general Japanese population: effects of age, gender, and physiologic characteristics. Circ J 71: 1452–1457.
31. Narumi T, Shishido T, Kiribayashi N, Kadowaki S, Nishiyama S, et al. (2012) Impact of insulin resistance on silent and ongoing myocardial damage in normal subjects: the Takahata study. Exp Diabetes Res 2012: 815098.
32. Pelsers MM, Chapelle JP, Knapen M, Vermeer C, Muijtjens AM, et al. (1999) Influence of age and sex and day-to-day and within-day biological variation on plasma concentrations of fatty acid-binding protein and myoglobin in healthy subjects. Clin Chem 45: 441–443.
33. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, et al. (1996) Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science 274: 1377–1379.
34. Makowski L, Boord JB, Maeda K, Babaev VR, Uysal KT, et al. (2001) Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. Nat Med 7: 699–705.
35. Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, et al. (2005) Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. Cell Metab 1: 107–119.
36. Furuhashi M, Fucho R, Gorgun CZ, Tuncman G, Cao H, et al. (2008) Adipocyte/macrophage fatty acid-binding proteins contribute to metabolic deterioration through actions in both macrophages and adipocytes in mice. J Clin Invest 118: 2640–2650.
37. Furuhashi M, Tuncman G, Gorgun CZ, Makowski L, Atsumi G, et al. (2007) Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. Nature 447: 959–965.
38. von Eynatten M, Breitling LP, Roos M, Baumann M, Rothenbacher D, et al. (2012) Circulating adipocyte fatty acid-binding protein levels and cardiovascular morbidity and mortality in patients with coronary heart disease: a 10-year prospective study. Arterioscler Thromb Vasc Biol 32: 2327–2335.
39. Maeda K, Uysal KT, Makowski L, Gorgun CZ, Atsumi G, et al. (2003) Role of the fatty acid binding protein mal1 in obesity and insulin resistance. Diabetes 52: 300–307.
40. Owada Y, Suzuki I, Noda T, Kondo H (2002) Analysis on the phenotype of E-FABP-gene knockout mice. Mol Cell Biochem 239: 83–86.
41. Hoekstra M, Stitzinger M, van Wanrooij EJ, Michon IN, Kruijt JK, et al. (2006) Microarray analysis indicates an important role for FABP5 and putative novel FABPs on a Western-type diet. J Lipid Res 47: 2198–2207.

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Dietary Patterns and Incident Functional Disability in Elderly Japanese: The Ohsaki Cohort 2006 Study

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Background. To date, little is known about the association between dietary pattern and disability in older adults. The present prospective cohort study investigated the association between dietary patterns and incident functional disability.

Methods. Information on food consumption and other lifestyle factors was collected from Japanese older persons aged ≥65 years via a questionnaire. Three dietary patterns (Japanese pattern, animal food pattern, and high dairy pattern) were derived using principal component analysis of the consumption of 39 food and beverage items. Data on functional disability were retrieved from the public Long-term Care Insurance database, in which participants were followed up for 5 years. The Cox model was used to estimate the multivariate-adjusted hazard ratios of incident functional disability.

Results. Among 14,260 participants, the 5-year incidence of functional disability was 16.6%. The Japanese pattern score was associated with a lower risk of incident functional disability (hazard ratio of the highest quartile vs the lowest, 0.77; 95% confidence interval: 0.68–0.88; p trend <.001). An animal food pattern and a high dairy pattern tended to have a higher risk of incident functional disability, but not to a significant degree.

Conclusions. In Japanese older persons, the Japanese dietary pattern is associated with a decreased risk of incident functional disability.

Key Words: Epidemiology—Functional performance—Nutrition.

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DIETARY patterns are widely employed in studies of the relationship between diet and health (1–3). Previously, epidemiological observations have indicated that the Mediterranean diet and the Japanese diet are associated with health benefits such as lower rates of mortality, cardiovascular disease, and depression (4–9).

With the aging of the populations of developed countries, a rapid increase in the proportion of elderly individuals with disability is imposing a large burden on social security systems worldwide (10). To date, three studies about the Mediterranean diet and three studies about the healthy dietary pattern (Healthy Eating Index) have investigated the association with the risk of functional decline (11–16). However, their sample sizes were not large, and only one study had a prospective design. Additionally, to our knowledge, the association between other dietary patterns and the risk of functional limitation has never been reported.

Japan has not only the longest life expectancy (79 years for men and 86 years for women), but also the longest healthy life expectancy (73 years for men and 78 years for women) of any country in the world (17). This may be partly attributable to the dietary patterns of the Japanese population, in particular, the Japanese diet. Therefore, the aim of the present analysis was to determine the association between dietary patterns and incident functional disability in elderly individuals in Japan.

Methods

Study Cohort

The design of the Ohsaki Cohort 2006 Study has been described in detail elsewhere (18). In brief, the source population for the baseline survey comprised all older citizens in Ohsaki City, Miyagi Prefecture, northeastern Japan, on December 1, 2006; 31,694 men and women aged ≥65 years. The survey included questions about the frequency of recent average consumption of 39 food items, as well as items on history of disease, blood pressure, education level, smoking, alcohol drinking, body weight, height, psychological distress score (K6) (19,20), time spent walking per day, and motor function score of the Kihon Checklist (21).

The baseline survey was conducted between December 1, 2006 and December 15, 2006. A questionnaire was distributed by the heads of individual administrative districts, and then collected by mail. For this analysis, 23,091 individuals

who provided valid responses formed the study cohort. We excluded 6,333 individuals who did not provide written consent for review of their Long-term Care Insurance (LTCI) information, 1,979 persons who had already been certified as having a disability by the LTCI at the time of the baseline survey, five persons who had died or moved during the period of the baseline survey, and 514 persons who left blank more than 24 of the 39 food items on the food frequency questionnaire (FFQ). Thus, 14,260 responses were analyzed for the purpose of this study.

During the 5-year period covered by the study, only 121 individuals were lost to follow-up because they moved away from the study area, without developing any functional disability; thus, the follow-up rate was 99.2%. From 62,755 person-years, incident functional disability was determined in 2,360 persons, and the number of all-cause deaths without incident functional disability was 842.

Dietary Assessment

We asked about the average frequency of consumption of each food using a 39-item FFQ, for which we had previously conducted a validation study in the same region (the precinct of Ohsaki Public Health Center, Miyagi) (22). In brief, 113 participants (55 men and 58 women) provided four 3-day diet records within a 1-year period and subsequently responded to the FFQ. The method used for calculating food and nutrient intake from the FFQ was developed in this study. Based on these data, we calculated the volume of consumption of individual foods according to the FFQ. For estimation of energy and protein intake from the food consumption volume based on the FFQ, we used a food composition table that corresponded to the items listed in the questionnaire. This food composition table was developed by using the Standard Tables of Food Composition published by the Science and Technology Agency of Japan (22).

Dietary Pattern Derivation

To derive dietary patterns, we used two methods: (i) factor analysis (principal component analysis) and (ii) confirmatory factor analysis.

The factor analysis was conducted by using the daily consumption (weight in grams) of 39 food items from the FFQ. If the reported frequency was blank, we assumed that the item was never consumed. We used the PROC FACTOR procedure in SAS version 9.3 to obtain a three-factor score. To achieve a simpler structure with greater interpretability, the factors were rotated by an orthogonal transformation (varimax rotation function in SAS). This allowed three major dietary patterns to be identified. We named them (i) the Japanese pattern, (ii) the animal food pattern, and (iii) the high dairy pattern. For each pattern and each participant, we calculated a factor score by summing the consumption of each food item weighted by its factor loading.

In order to strengthen our dietary pattern analysis, we further used confirmatory factor analysis, which is characterized by hypothesis-oriented approach. Recently, confirmatory factor analysis has been used increasingly as a major analytical method in dietary pattern research, such as studies of the Mediterranean diet (23–25). We identified nine food items that formed the Japanese Diet Index Score: rice, miso soup, seaweeds, pickles, green and yellow vegetables (green vegetables, carrot, pumpkin, tomato), fish (raw fish, fish boiled with soy, roast fish, boiled fish paste, dried fish), green tea, beef and pork (beef, pork, ham, sausage), and coffee. In a previous study based on the dietary record method, these items had been reported to have higher absolute factor scores for the traditional Japanese pattern (26). Another study has also reported that these items are characteristic of the traditional Japanese diet (27). For each of the seven positive components (rice, miso soup, seaweeds, pickles, green and yellow vegetables, fish, and green tea), participants received 1 point if their intake was more than or equal to the sex-specific median. For each of the two negative components (beef and pork, and coffee), participants received 1 point if their intake was below the sex-specific median. Thus, the Japanese Diet Index Score ranged from 0 to 9, with higher scores indicating greater dietary conformity.

Covariate

Body mass index was calculated as the self-reported body weight (kg) divided by the square of the self-reported body height (m).

The K6 was used as an indicator of psychological distress (19,20). Respondents were asked about their mental status over the last month by using six questions. Total point scores ranged from 0 to 24. As the optimal cutoff point for mental illness in the validation study, we classified individuals with scores of ≥13 as having psychological distress (20).

The Kihon Checklist was developed to predict functional decline in community-dwelling elderly individuals. With regard to the motor function score in the Kihon Checklist, respondents were asked about their current motor function status by using five binary questions yielding total point scores ranging from 0 to 5. As the optimal cutoff point for functional decline suggested in the validation study, we classified individuals with scores of <3 as having better motor function (21).

LTCI System in Japan

In this study, we defined incident functional disability as certification for LTCI in Japan, which uses a nationally uniform standard of functional disability. LTCI is a mandatory form of social insurance to assist daily activity in the frail elderly individuals (28–32). Everyone aged ≥40 years pays premiums, and everyone aged ≥65 years is eligible for

formal caregiving services. When a person applies to the municipal government for benefits, a care manager visits his or her home and assesses the degree of functional disability using a questionnaire developed by the Ministry of Health, Labor, and Welfare. Then, the municipal government calculates the standardized scores for physical and mental functions on the basis of the questionnaire and assesses whether the applicant is eligible for LTCI benefits (certification). There are a total of 74 items in the questionnaire, and these are classified into six dimensions: motor function (13 items), activity of daily living (12 items), cognitive function (9 items), mental and behavioral disorders (15 items), adaptation to social life (6 items), and use of medical procedures (12 items). If a person is judged to be thus eligible, the Municipal Certification Committee decides on one of seven levels of support, ranging from Support Level 1, Support Level 2, and Care Level 1 to Care Level 5. In brief, LTCI certification levels are defined as follows. Support Level 1: "limited in instrumental activities of daily living but independent in basic activities of daily living (ADLs)"; Care Level 2: "requiring assistance in at least one basic ADL task"; Care Level 5: "requiring care in all ADL tasks". A community-based study has shown that levels of LTCI certification are well correlated with ability to perform activities of daily living, and with Mini-Mental State Examination scores (33). LTCI certification has already been used as a measure of incident functional disability in the elderly individuals (34–36).

Follow-up and Case Details

Incident functional disability was set as our endpoint, which was defined as LTCI certification. The primary outcome was LTCI certification (Support Level 1 or higher), in which deaths without LTCI certification were treated as censored. In the subanalysis, we set the criteria of disability toward a more severe level, that is, Care Level 2 (requiring assistance with one basic activities of daily living task) or higher.

We obtained a data set that included information on the date of LTCI certification, death, or emigration from Ohsaki City Government based on an agreement about the secondary use of data. With regard to LTCI certification, information on care level was also provided. All data were transferred from the Ohsaki City Government under the agreement related to Epidemiologic Research and Privacy Protection yearly each December.

Ethical Issues

We considered the return of completed questionnaires to imply consent to participate in the study involving the baseline survey data and subsequent follow-up of death and emigration. We also confirmed information regarding LTCI certification status after obtaining written consent along with the questionnaires returned from the participants at the time of the baseline survey. The Ethics Committee of Tohoku University Graduate School of Medicine (Sendai, Japan) reviewed and approved the study protocol.

Statistical Analysis

We counted the person-years of follow-up for each participant from December 16, 2006 until the date of incident functional disability, date of emigration from Ohsaki City, date of death, or the end of the study period (November 30, 2011), whichever occurred first.

We used the multiple adjusted Cox proportional hazard model to calculate the hazard ratios (HRs) and 95% confidence intervals (CIs) for incident functional disability according to quartiles of the dietary pattern score. Dummy variables were created for the quartiles of each dietary pattern score, and the lowest quartile of a dietary pattern score was used as a reference category. Multivariate models were adjusted for the following variables. Model 1 was sex and age adjusted. To examine whether the association between the dietary patterns and functional disability was attributable to a healthy physical status or other lifestyle factors, Model 2 was further adjusted for history of stroke, myocardial infarction, hypertension (individuals with self-measured systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg were also defined as hypertensive), arthritis, osteoporosis and fracture, education level, smoking status, alcohol consumption, body mass index, psychological distress score, time spent walking per day, and motor function score. Model 3 was fully adjusted and included energy and protein intake (category of sex-specific tertile).

All data were analyzed using SAS version 9.3 (SAS Inc., Cary, NC). All statistical tests described here were two sided, and differences at $p < .05$ were accepted as significant.

Results

Among 14,260 participants, the proportion of men was 44.8%, mean (*SD*) age was 73.9 (6.0) years, and mean (*SD*) body mass index was 23.6 (3.4). The number of participants for whom data on the FFQ were any missing was 7,352 (the distribution of missing shows in Supplementary Figure 1).

Table 1 shows factor loadings, which are equivalent to simple correlations between the food items and dietary patterns. A positive loading indicates that a food item is positively associated with the dietary pattern, and a negative loading indicates an inverse association with the dietary pattern. That is, food items highly loaded within a dietary pattern are highly correlated with each other.

The Japanese pattern was loaded heavily on fish, vegetables, mushrooms, potato, seaweeds, pickles, soybean and fruits, whereas the animal food pattern was loaded heavily on various animal-derived foods (beef, pork, ham, sausage, chicken, liver, eggs, and butter). The high dairy pattern was heavily loaded on dairy products (yoghurt, cheeses, and butter), margarine, and

Table 1. Factor-Loading Matrix for the Major Dietary Pattern Identified by Factor Analysis (n = 14,260)*

	Japanese Pattern	Animal Food Pattern	High Dairy Pattern
Rice	0.01	0.31	−0.39
Miso soup	0.06	0.04	−0.07
Beef	−0.09	0.47	0.08
Pork (excluding ham, sausage)	0.15	0.56	0.01
Ham, sausage	0.05	0.49	0.25
Chicken	0.15	0.56	0.00
Liver	−0.06	0.42	0.15
Egg	0.25	0.33	0.04
Milk	0.19	0.01	0.26
Yoghurt	0.21	−0.09	0.47
Cheeses	−0.02	0.28	0.46
Butter	−0.07	0.39	0.45
Margarine	−0.07	0.23	0.49
Deep fried dishes, tempura	0.15	0.47	0.04
Fried vegatable	0.40	0.32	0.02
Raw fish, fish boiled with soy, roast fish	0.44	0.26	−0.11
Boiled fish paste	0.29	0.38	0.15
Dried fish	0.33	0.30	0.05
Green vegetables	0.66	0.10	0.08
Carrot, pumpkin	0.65	−0.03	0.23
Tomato	0.44	−0.12	0.37
Cabbage, lettuce	0.60	0.17	0.16
Chinese cabbage	0.64	0.15	−0.05
Wild plant	0.19	0.22	0.16
Mushrooms (shiitake, enokitake)	0.43	0.27	0.04
Potato	0.65	0.07	0.12
Seaweeds	0.63	0.02	0.19
Pickles (radish, Chinese cabbage)	0.41	0.04	−0.06
Food boiled with soy	0.23	0.23	0.32
Boiled beans	0.22	0.11	0.37
Soybean (tofu, fermented soybeans)	0.56	0.11	−0.04
Orange	0.54	−0.12	0.34
Other fruits	0.54	−0.13	0.39
Fresh juice	0.13	0.13	0.37
Confectioneries	0.26	0.16	0.17
Green tea	0.28	0.02	0.04
Black tea	0.03	0.09	0.37
Coffee	0.05	0.13	0.18
Chinese tea	0.01	0.04	0.25
Variance explained (%)	12.8	7.1	6.3

*Absolute values <−0.3 or >0.3 are underlined.

black tea, and negatively loaded on rice. These three dietary patterns explained 26.1% of the variance.

Table 2 compares the characteristics of participants according to the quartiles of each dietary pattern score. Participants with a higher Japanese pattern score were less likely to be male, to be current smokers and drinkers, and to suffer from psychological distress. Additionally, participants with a higher Japanese pattern score were more likely to have ≥19 years of education, to walk ≥1 h/d, and to have greater intake of energy and protein. Conversely, participants with a higher animal food pattern score were more likely to be male, to be current smokers and drinkers. Additionally, participants with a higher animal food pattern score were more likely to walk ≥1 h/d, to have better motor function, and to have greater intake of energy and protein, and were less likely to suffer from psychological distress and to have ≥19 years of education. Participants with a high dairy pattern score tended to be female, and were similar to those with a higher Japanese dietary pattern score except for psychological distress, time spent walking, and intake of energy and protein.

The association between the dietary patterns and functional disability, along with HRs and associated 95% CIs, are shown in Table 3. We found that a higher Japanese pattern score was inversely associated with the incident risk of functional disability (p trend <.001 in Model 3). This inverse association did not differ between the sexes (p = .057 for interaction with sex; data not shown). On the other hand, the animal food pattern and the high dairy pattern were not significantly associated with functional disability in any of the models.

Even when we set stricter criteria for disability (basic activities of daily living impairment), the results for the

Table 2. Baseline Characteristics According to Dietary Pattern Score Quartiles (n = 14,260)

Characteristics	Dietary Pattern Score Quartiles			
	1 (Low)	2	3	4 (High)
Age (y), mean (*SD*)				
Japanese pattern	74.0 (6.3)	73.7 (5.9)	73.6 (5.8)	74.3 (5.8)
Animal food pattern	74.0 (6.2)	73.9 (6.0)	73.7 (5.8)	74.0 (5.9)
High dairy pattern	74.3 (5.9)	74.2 (6.1)	73.7 (5.9)	73.4 (5.9)
Men (%)				
Japanese pattern	56.8	49.1	39.1	34.5
Animal food pattern	19.9	36.8	52.6	70.1
High dairy pattern	67.8	45.3	34.6	31.7
Education until age ≥19 y (%)				
Japanese pattern	21.6	25.8	29.2	31.7
Animal food pattern	30.3	26.6	25.5	26.1
High dairy pattern	16.2	23.4	30.0	38.7
Current smoker (%)				
Japanese pattern	20.3	15.1	10.3	7.4
Animal food pattern	6.9	11.6	15.2	19.4
High dairy pattern	21.5	12.5	10.1	9.0
Current drinker (%)				
Japanese pattern	43.2	41.2	36.1	30.2
Animal food pattern	23.4	33.4	41.1	52.2
High dairy pattern	49.6	36.2	32.9	31.9
Body mass index (kg/m^2), mean (*SD*)				
Japanese pattern	23.6 (3.5)	23.5 (3.4)	23.6 (3.3)	23.7 (3.2)
Animal food pattern	23.6 (3.4)	23.6 (3.5)	23.6 (3.2)	23.5 (3.2)
High dairy pattern	23.6 (3.3)	23.6 (3.4)	23.6 (3.4)	23.6 (3.4)
Psychological distress (%)*				
Japanese pattern	7.3	4.7	4.1	3.1
Animal food pattern	6.3	4.4	4.2	4.2
High dairy pattern	4.3	5.5	4.7	4.5
Time spent walking ≥1 h/d (%)				
Japanese pattern	24.6	24.7	28.1	31.9
Animal food pattern	23.1	26.0	28.1	32.2
High dairy pattern	31.5	26.7	26.7	24.5
Better motor function (%)†				
Japanese pattern	75.8	77.8	78.6	78.4
Animal food pattern	73.3	76.4	78.7	82.2
High dairy pattern	77.7	76.1	77.3	79.5
Energy (kcal), mean (*SD*)‡				
Japanese pattern	1,214 (395)	1,384 (366)	1,484 (365)	1,614 (374)
Animal food pattern	1,188 (301)	1,336 (322)	1,493 (344)	1,735 (409)
High dairy pattern	1,573 (436)	1,390 (393)	1,367 (379)	1,428 (368)
Protein (g), mean (*SD*)				
Japanese pattern	40.8 (12.1)	50.2 (11.3)	56.4 (11.5)	63.6 (12.2)
Animal food pattern	44.1 (11.7)	49.1 (11.7)	55.2 (11.3)	65.6 (13.1)
High dairy pattern	55.4 (14.1)	50.7 (14.6)	51.8 (14.1)	55.8 (14.1)

*Kessler six-item psychological distress scale ≥13.
†Motor function score in Kihon Checklist <3.
‡Except energy intake from alcohol drinking.

Japanese pattern score did not change. In Model 3, the multivariate HRs (95% CI) for the successive categories of the Japanese pattern score were: 1 (reference), 0.87 (0.73–1.03), 0.71 (0.59–0.86), and 0.74 (0.61–0.90) (p trend <.001; data not shown). To examine possible reverse causality, we reanalyzed the association after excluding 900 participants who experienced incident functional disability in the first 2 years of follow-up, but the results did not change substantially in Model 3 (p trend <.001; data not shown). Additionally, when we excluded participants who had any history of disease that might have affected dietary habit (stroke, myocardial infarction, diabetes, arthritis, osteoporosis, fracture, cancer, kidney disease, and hepatic disease), the results did not change substantially in Model 3 (p trend = .007; data not shown). The Japanese pattern score was also inversely associated with all-cause mortality (p trend = .028 in Model 3; data not shown).

Table 4 shows the results of incident functional disability according to the quartiles of the Japanese Diet Index Score. In this analysis, we included 514 persons who left

Table 3. Association Between Dietary Pattern Scores and Incident Functional Disability (n = 14,260)

	Dietary Pattern Score Quartiles				
	1 (Low)	2	3	4 (High)	p trend
Japanese pattern					
Number of event	711	591	522	536	
Person-years	15,159	15,649	15,948	15,999	
Model 1*	1.00 (reference)†	0.83 (0.75–0.93)	0.72 (0.64–0.81)	0.65 (0.58–0.72)	<.001
Model 2‡	1.00 (reference)	0.90 (0.80–1.00)	0.80 (0.71–0.89)	0.75 (0.67–0.84)	<.001
Model 3§	1.00 (reference)	0.91 (0.82–1.02)	0.82 (0.73–0.92)	0.77 (0.68–0.88)	<.001
Animal food pattern					
Number of event	616	620	557	567	
Person-years	15,601	15,730	15,767	15,657	
Model 1*	1.00 (reference)	1.04 (0.93–1.16)	0.97 (0.86–1.09)	1.00 (0.88–1.13)	.697
Model 2‡	1.00 (reference)	1.08 (0.96–1.21)	1.03 (0.91–1.16)	1.09 (0.96–1.23)	.313
Model 3 §	1.00 (reference)	1.10 (0.98–1.23)	1.07 (0.95–1.21)	1.16 (1.02–1.31)	.053
High dairy pattern					
Number of event	615	611	558	576	
Person-years	15,502	15,618	15,905	15,730	
Model 1*	1.00 (reference)	0.96 (0.85–1.07)	0.89 (0.79–1.00)	0.98 (0.87–1.10)	.465
Model 2‡	1.00 (reference)	0.99 (0.89–1.11)	0.95 (0.84–1.07)	1.10 (0.98–1.24)	.217
Model 3§	1.00 (reference)	0.99 (0.88–1.11)	0.95 (0.84–1.07)	1.11 (0.99–1.26)	.158

*Adjusted for age (65–69, 70–74, 75–79, 80–84, and ≥85 y) and sex.

†HR (95% CI).

‡Adjusted for model 1 + history of disease (stroke, myocardial infarction, hypertension, arthritis, osteoporosis, fracture [yes, no]), educational level (age when last graduation of school <16, 16–18, ≥19 y, missing), smoking (never, former, current, missing), alcohol drinking (never, former, current, missing), body mass index (in kg/m^2: <18.5, 18.5–24.9, ≥25.0, missing), psychological distress score (<13, ≥13, missing), time spent walking (<30 min/d, 30 min to 1 h/d, ≥1 h/d, missing), and motor function score (<3, ≥3, missing).

§Adjusted for model 2 + tertile categories of energy intake and protein intake (sex-specific tertile, missing).

Table 4. Confirmatory Factor Analysis: Association Between Japanese Diet Index Score and Incident Functional Disability (n = 10,148)

	Japanese Diet Index Score (quartiles)*				
	1 (Low)	2	3	4 (High)	p trend
Index score	<4	4	5	≥6	
Number of event	374	333	374	481	
Person-years	9,793	9,293	10,661	15,261	
Model 1†	1.00 (reference)‡	0.88 (0.76–1.02)	0.82 (0.71–0.94)	0.72 (0.63–0.83)	<.001
Model 2§	1.00 (reference)	0.92 (0.79–1.07)	0.87 (0.76–1.01)	0.77 (0.67–0.88)	<.001
Model 3‖	1.00 (reference)	0.94 (0.81–1.09)	0.90 (0.77–1.05)	0.79 (0.68–0.92)	.002

*Index score was constituted by nine food items that reported to have higher absolute factor scores for the traditional Japanese pattern. For each of the seven positive components (rice, miso soup, seaweeds, pickles, green and yellow vegetables, fish, and green tea), participants received 1 point if their intake was more than or equal to the sex-specific median. For each of the two negative components (beef and pork and coffee), participants received 1 point if their intake was below the sex-specific median.

†Adjusted for age (65–69, 70–74, 75–79, 80–84, and ≥85 y) and sex.

‡HR (95% CI).

§Adjusted for model 1 + history of disease (stroke, myocardial infarction, hypertension, arthritis, osteoporosis, fracture [yes, no]), educational level (age when last graduation of school <16, 16–18, ≥19 y, missing), smoking (never, former, current, missing), alcohol drinking (never, former, current, missing), body mass index (in kg/m^2: <18.5, 18.5–24.9, ≥25.0, missing), psychological distress score (<13, ≥13, missing), time spent walking (<30 min/d, 30 min to 1 h/d, ≥1 h/d, missing), and motor function score (<3, ≥3, missing).

‖Adjusted for model 2 + tertile categories of energy intake and protein intake (sex-specific tertile, missing).

blank more than 24 items on the FFQ and then excluded 4,626 persons for whom data on items of the Japanese Diet Index Score were missing (10,148 persons were included in the analysis). We found a significant inverse association between the Japanese Diet Index Score and functional disability (p trend = .002 in Model 3).

Discussion

In this population-based cohort study, we identified three dietary patterns derived by factor analysis among the Japanese population: the Japanese pattern, animal food pattern, and high dairy pattern, which were consistent with our previous study using the same FFQ, except for the third pattern because in the present study the volume of alcohol consumption was not available (6). The Japanese pattern was associated with a decreased risk of incident functional disability. No apparent association was observed for either the animal food pattern or the high dairy pattern. To our knowledge, this is the first study to have proved the association between the Japanese dietary pattern and incident risk of functional disability.

Our study had a number of strengths: (i) it was a large population-based cohort study of 14,260 persons; (ii) it had a follow-up rate of almost 100%; (iii) many confounding factors were taken into account.

We also considered the effects of reverse causality. Even after excluding individuals who experienced incident functional disability in the first 2 years of follow-up, the strong inverse association between the Japanese pattern and functional disability persisted. The earlier findings suggest that the present results are unlikely to be explained by reverse causality.

This inverse association between the Japanese pattern and functional disability was consistent with previous studies of the Mediterranean diet and Healthy Eating Index (11–16). The Japanese pattern has some characteristics in common with the Mediterranean diet, for instance, high intake of vegetables, fruits, legumes, and fish, and low intake of meat and dairy products (4,37). Thus, the mechanism of this association might be similar to that reported in the previous studies of the Mediterranean diet. On the other hand, the Healthy Eating Index pattern in three previous studies may not be fully consistent with Japanese pattern because meat, but not fish, was recommended in the Healthy Eating Index. Furthermore, vegetables, fruits, and legumes are common components of the above three patterns. Previous studies have reported that a plant-based diet reduces cardiovascular risk, type 2 diabetes, and bone loss (37–42). Although a diet consisting only of plant-based foods may lack certain nutrients (42), a dietary pattern including an abundant amount of these plant-based items may decrease the risk of functional disability.

On the other hand, the Japanese pattern is reported to differ from the Mediterranean diet in that energy intake is lower (43). The Japanese pattern score was positively correlated with energy intake (Table 2), but the results did not change substantially even when energy intake and body mass index were added in the multivariate model. The inverse association seems difficult to explain in terms of energy intake alone, and micronutritional components might have a role. Because the Japanese pattern included a variety of foods that explained 12.8% of the overall variance, this pattern may contain various micronutritional components and have a good nutrient balance.

In the present study, we used two different dietary pattern derivation methods to strengthen the reliability of our results. When we repeated the analysis by using confirmatory factor analysis, higher conformity with the Japanese Diet Index Score was also associated with a decreased risk of incident functional disability. Because these factors in the Japanese Diet Index Score were based on nationwide validity studies of dietary pattern, the Japanese Diet Index Score may represent the most common diet items in Japanese population. However, the Japanese diet also varies in several aspects according to region. For example, residents of Okinawa, who are known for their longer average life expectancy, often eat the traditional Okinawan diet that includes unique food items such as bitter melon, mugwort, and turmeric (44).

Additionally, in the analysis using each item from the Japanese Diet Index Score as an exposure variable (dichotomous variable by sex-specific median), a significant inverse association was observed for items other than fish and vegetables (data not shown). These results suggested that traditional Japanese foods were also associated with a decreased risk of incident functional disability. Previous studies have also examined the health impact of individual Japanese foods and their nutritional components, including soybeans (as well as miso soup), seaweed, and green tea (45–49).

This study had several limitations. First, we did not investigate the causes of functional disability in participants who received LTCI certification. Thus, the mechanism responsible for reduction of functional disability by intake of a Japanese diet remained unidentified. Second, not all potential confounding factors were considered, as we used only indirect measures of physical and cognitive function for adjustment. Third, because not all candidates applied for LTCI certification, this study may not have been completely free from detection bias. The degree of this bias remains to be verified.

In conclusion, the findings of this cohort study indicate that higher conformity with the Japanese dietary pattern is significantly associated with a lower risk of incident functional disability. This result suggests that the Japanese dietary pattern contributes to extended healthy life expectancy.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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Conflict of Interest

There are no potential conflicts of interest that relate to the manuscript.

References

1. Mente A, de Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Arch Intern Med.* 2009;169:659–669. doi:10.1001/archinternmed.2009.38
2. Bhupathiraju SN, Tucker KL. Coronary heart disease prevention: nutrients, foods, and dietary patterns. *Clin Chim Acta.* 2011;412:1493–1514. doi:10.1016/j.cca.2011.04.038
3. Brennan SF, Cantwell MM, Cardwell CR, Velentzis LS, Woodside JV. Dietary patterns and breast cancer risk: a systematic review and meta-analysis. *Am J Clin Nutr.* 2010;91:1294–1302. doi:10.3945/ajcn.2009.28796
4. Willett WC. Diet and health: what should we eat? *Science.* 1994;264:532–537. doi:10.1126/science.8160011
5. Truswell AS. Practical and realistic approaches to healthier diet modifications. *Am J Clin Nutr.* 1998;67:583S–590S.

6. Shimazu T, Kuriyama S, Hozawa A, et al. Dietary patterns and cardiovascular disease mortality in Japan: a prospective cohort study. *Int J Epidemiol*. 2007;36:600–609. doi:10.1093/ije/dym005
7. Nakamura Y, Ueshima H, Okamura T, et al. A Japanese diet and 19-year mortality: national integrated project for prospective observation of non-communicable diseases and its trends in the aged, 1980. *Br J Nutr*. 2009;101:1696–1705. doi:10.1017/S0007114508111503
8. Nanri A, Kimura Y, Matsushita Y, et al. Dietary patterns and depressive symptoms among Japanese men and women. *Eur J Clin Nutr*. 2010;64:832–839. doi:10.1038/ejcn.2010.86
9. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to Mediterranean diet and health status: meta-analysis. *BMJ*. 2008;337:a1344. doi:10.1136/bmj.a1344
10. Organisation for Economic Co-operation and Development (OECD). *Help Wanted? Providing and Paying for Long-Term Care: OECD Publishing*; 2011. http://www.oecd-ilibrary.org/social-issues-migration-health/help-wanted_9789264097759-en. Accessed August 12, 2013. doi:10.1787/9789264097759-en
11. Bollwein J, Diekmann R, Kaiser MJ, et al. Dietary quality is related to frailty in community-dwelling older adults. *J Gerontol A Biol Sci Med Sci*. 2013;68:483–489. doi:10.1093/gerona/gls204
12. Milaneschi Y, Bandinelli S, Corsi AM, et al. Mediterranean diet and mobility decline in older persons. *Exp Gerontol*. 2011;46:303–308. doi:10.1016/j.exger.2010.11.030
13. Talegawkar SA, Bandinelli S, Bandeen-Roche K, et al. A higher adherence to a Mediterranean-style diet is inversely associated with the development of frailty in community-dwelling elderly men and women. *J Nutr*. 2012;142:2161–2166. doi:10.3945/jn.112.165498
14. Xu B, Houston D, Locher JL, Zizza C. The association between Healthy Eating Index-2005 scores and disability among older Americans. *Age Ageing*. 2012;41:365–371. doi:10.1093/ageing/afr158
15. Xu B, Houston DK, Locher JL, et al. Higher Healthy Eating Index-2005 scores are associated with better physical performance. *J Gerontol A Biol Sci Med Sci*. 2012;67:93–99. doi:10.1093/gerona/glr159
16. Koster A, Penninx BW, Newman AB, et al. Lifestyle factors and incident mobility limitation in obese and non-obese older adults. *Obesity*. 2007;15:3122–3132. doi:10.1038/oby.2007.372
17. WHO. *World Health Statistics 2010*. Geneva: World Health Organization; 2010. http://www.who.int/whosis/whostat/2010/en/. Accessed August 12, 2013.
18. Kuriyama S, Nakaya N, Ohmori-Matsuda K, et al. The Ohsaki Cohort 2006 Study: design of study and profile of participants at baseline. *J Epidemiol*. 2010;20:253–258. doi:10.2188/jea.JE20090093
19. Kessler RC, Andrews G, Colpe LJ, et al. Short screening scales to monitor population prevalences and trends in non-specific psychological distress. *Psychol Med*. 2002;32:959–976. doi:10.1017/S0033291702006074
20. Kessler RC, Barker PR, Colpe LJ, et al. Screening for serious mental illness in the general population. *Arch Gen Psychiatry*. 2003;60:184–189. doi:10.1001/archpsyc.60.2.184
21. Tomata Y, Hozawa A, Ohmori-Matsuda K, et al. Validation of the Kihon Checklist for predicting the risk of 1-year incident long-term care insurance certification: the Ohsaki Cohort 2006 Study. *Nippon Koshu Eisei Zasshi*. 2011;58:3–13 (in Japanese).
22. Ogawa K, Tsubono Y, Nishino Y, et al. Validation of a food-frequency questionnaire for cohort studies in rural Japan. *Public Health Nutr*. 2003;6:147–157. doi:10.1079/PHN2002411
23. Féart C, Samieri C, Rondeau V, et al. Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. *JAMA*. 2009;302:638–648. doi:10.1001/jama.2009.1146
24. Mitrou PN, Kipnis V, Thiébaut AC, et al. Mediterranean dietary pattern and prediction of all-cause mortality in a US population: results from the NIH-AARP Diet and Health Study. *Arch Intern Med*. 2007;167:2461–2468. doi:10.1001/archinte.167.22.2461
25. Sánchez-Villegas A, Delgado-Rodríguez M, Alonso A, et al. Association of the Mediterranean dietary pattern with the incidence of depression: the Seguimiento Universidad de Navarra/University of Navarra follow-up (SUN) cohort. *Arch Gen Psychiatry*. 2009;66:1090–1098. doi:10.1001/archgenpsychiatry.2009.129
26. Okubo H, Murakami K, Sasaki S, et al. Relative validity of dietary patterns derived from a self-administered diet history questionnaire using factor analysis among Japanese adults. *Public Health Nutr*. 2010;13:1080–1089. doi:10.1017/S1368980009993211
27. Nanri A, Shimazu T, Ishihara J, et al. Reproducibility and validity of dietary patterns assessed by a food frequency questionnaire used in the 5-year follow-up survey of the Japan Public Health Center-Based Prospective Study. *J Epidemiol*. 2012;22:205–215. doi:10.2188/jea.JE20110087
28. Imai H, Fujii Y, Fukuda Y, Nakao H, Yahata Y. Health-related quality of life and beneficiaries of long-term care insurance in Japan. *Health Policy*. 2008;85:349–355. doi:10.1016/j.healthpol.2007.08.008
29. Ikegami N. Public long-term care insurance in Japan. *JAMA*. 1997;278:1310–1314. doi:10.1001/jama.1997.03550160030017
30. Tsutsui T, Muramatsu N. Care-needs certification in the long-term care insurance system of Japan. *J Am Geriatr Soc*. 2005;53:522–527. doi:10.1111/j.1532-5415.2005.53175.x
31. Imahashi K, Kawagoe M, Eto F, Haga N. Clinical status and dependency of the elderly requiring long-term care in Japan. *Tohoku J Exp Med*. 2007;212:229–238. doi:10.1620/tjem.212.229
32. Ministry of Health, Labor, and Welfare. *Long-Term Care, Health and Welfare Services for the Elderly*. http://www.mhlw.go.jp/english/policy/care-welfare/care-welfare-elderly/. Accessed August 12, 2013.
33. Arai Y, Zarit SH, Kumamoto K, Takeda A. Are there inequities in the assessment of dementia under Japan's LTC insurance system? *Int J Geriatr Psychiatry*. 2003;18:346–352. doi:10.1002/gps.836
34. Hozawa A, Sugawara Y, Tomata Y, et al. Relationship between serum isoflavone levels and disability-free survival among community-dwelling elderly individuals: nested case-control study of the Tsurugaya project. *J Gerontol A Biol Sci Med Sci*. 2013;68:465–472. doi:10.1093/gerona/gls198
35. Kondo N, Kawachi I, Hirai H, et al. Relative deprivation and incident functional disability among older Japanese women and men: prospective cohort study. *J Epidemiol Community Health*. 2009;63:461–467. doi:10.1136/jech.2008.078642
36. Tomata Y, Kakizaki M, Nakaya N, et al. Green tea consumption and the risk of incident functional disability in elderly Japanese: the Ohsaki Cohort 2006 Study. *Am J Clin Nutr*. 2012;95:732–739. doi:10.3945/ajcn.111.023200
37. Trichopoulou A, Naska A, Antoniou A, Friel S, Trygg K, Turrini A. Vegetable and fruit: the evidence in their favour and the public health perspective. *Int J Vitam Nutr Res*. 2003;73:63–69. doi:10.1024/0300-9831.73.2.63
38. Mishra S, Xu J, Agarwal U, Gonzales J, Levin S, Barnard ND. A multicenter randomized controlled trial of a plant-based nutrition program to reduce body weight and cardiovascular risk in the corporate setting: the GEICO study. *Eur J Clin Nutr*. 2013;67:718–724. doi:10.1038/ejcn.2013.92
39. Hu FB. Plant-based foods and prevention of cardiovascular disease: an overview. *Am J Clin Nutr*. 2003;78:544S–551S.
40. de Koning L, Chiuve SE, Fung TT, Willett WC, Rimm EB, Hu FB. Diet-quality scores and the risk of type 2 diabetes in men. *Diabetes Care*. 2011;34:1150–1156. doi:10.2337/dc10-2352
41. Anderson JJ. Plant-based diets and bone health: nutritional implications. *Am J Clin Nutr*. 1999;70(suppl 3):539S–542S.
42. McEvoy CT, Temple N, Woodside JV. Vegetarian diets, low-meat diets and health: a review. *Public Health Nutr*. 2012;15:2287–2294. doi:10.1017/S1368980012000936
43. Tokudome S, Ichikawa Y, Okuyama H, et al. The Mediterranean vs the Japanese diet. *Eur J Clin Nutr*. 2004;58:1323. doi:10.1038/sj.ejcn.1601970

44. Willcox DC, Willcox BJ, Todoriki H, Suzuki M. The Okinawan diet: health implications of a low-calorie, nutrient-dense, antioxidant-rich dietary pattern low in glycemic load. *J Am Coll Nutr*. 2009;28(suppl):500S–516S.
45. Tada N, Maruyama C, Koba S, et al. Japanese dietary lifestyle and cardiovascular disease. *J Atheroscler Thromb*. 2011;18:723–734. doi:10.5551/jat.8193
46. Messina M. A brief historical overview of the past two decades of soy and isoflavone research. *J Nutr*. 2010;140:1350S–1354S. doi:10.3945/jn.109.118315
47. Yoshinaga M, Toda N, Tamura Y, et al. Japanese traditional miso soup attenuates salt-induced hypertension and its organ damage in Dahl salt-sensitive rats. *Nutrition*. 2012;28:924–931. doi:10.1016/j.nut.2011.09.010
48. MacArtain P, Gill CI, Brooks M, Campbell R, Rowland IR. Nutritional value of edible seaweeds. *Nutr Rev*. 2007;65:535–543. doi:10.1111/j.1753-4887.2007.tb00278.x
49. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea–a review. *J Am Coll Nutr*. 2006;25:79–99.

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Total and high molecular weight adiponectin levels and risk of cardiovascular disease in individuals with high blood glucose levels

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Prospective study

ABSTRACT

Objective: The association of adiponectin levels with cardiovascular disease (CVD) may vary by age and health condition. It is unknown whether adiponectin predicts CVD events among individuals with high blood glucose levels.
Methods: We conducted a nested case–control study among 15,566 men and women aged 40–85 years from four communities, who were free of CVD at baseline. During 192,181 person-years of follow-up, 117 individuals subsequently developed coronary heart disease or ischemic stroke and had high plasma glucose concentrations (fasting/nonfasting ≥ 5.6/7.2 mmol/L or treated) at baseline. Controls were randomly selected at a 2:1 ratio and matched for sex, age, blood glucose, year of survey, fasting conditions, and community ($n = 234$). Baseline total and high molecular weight (HMW) adiponectin and their ratio were examined for total subjects and the association with CVD was compared between ages of 40–69 and 70–85 years.
Results: After adjustment for matched variables and traditional risk factors, total and HMW adiponectin and their ratio were not associated with overall risk of CVD. However, significant interactions of the associations between the age groups were found. The highest quartile for HMW adiponectin and HMW/total adiponectin ratio decreased risk of CVD compared with the lowest quartile among middle-aged individuals (multivariable-adjusted odds ratio = 0.33 [95%CI, 0.13–0.83] and 0.47 [0.22–0.98], respectively), while this association was not seen among the elderly.
Conclusions: High HMW adiponectin levels may decrease the risk of CVD in middle-aged adults with high blood glucose.

1. Introduction

Adiponectin is an adipocyte-derived, anti-atherogenic protein that is found as a trimer, hexamer, or as a high-molecular weight (HMW) complex (12–18 subunits) in human plasma, and which plays a role in the regulation of glucose and lipid metabolism [1,2]. Treatment with recombinant full-length adiponectin has been shown to improve insulin resistance, hyperglycemia, and hyperlipidemia in adiponectin-deficient mice [3]. Furthermore, the HMW form of adiponectin was shown to selectively improve insulin resistance, and a low ratio of HMW to total adiponectin was found to be a useful predictor of insulin resistance and metabolic syndrome in clinical studies [4].

However, epidemiological evidence for a protective role of adiponectin against the development of type 2 diabetes and CVD has been limited and inconsistent [5]. Several prospective cohort studies showed that lower adiponectin levels were associated with elevated risk of new onset of type 2 diabetes [6] and incident

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coronary heart disease [7]. In contrast, other studies have reported weak or non-significant associations between low adiponectin levels and cardiovascular disease (CVD) [8], particularly among women [9,10] and the elderly [11]. Furthermore, high adiponectin levels have been associated with an increased risk of CVD among elderly people [12–14].

Taking into consideration the stronger association between glucose abnormalities and CVD events among middle-aged adults than in the elderly [15], we hypothesized that adiponectin levels might also be more strongly associated with CVD events in middle-aged adults than in the elderly. We examined HMW adiponectin levels and the HMW/total adiponectin ratio, which have been shown to be more closely related to insulin sensitivity than total adiponectin [4]. We also hypothesized that adiponectin levels might be more predictive of CVD among individuals with high blood glucose levels, since fasting and non-fasting blood glucose levels were shown to be associated with an increased risk of coronary heart disease and stroke in Japanese men and women aged 40–69 years [16,17]. In the present nested case–control study, we attempted to clarify the association between adiponectin and risk of ischemic stroke and coronary heart disease events among individuals with high blood glucose levels using 12.3 years of follow-up data from approximately 16,000 individuals.

2. Methods

2.1. Study population

The Circulatory Risk in Communities Study (CIRCS) [18] and Ozu Study [19] were prospective cohort studies of Japanese men and women aged 40 years and older in communities across Japan. Baseline surveys were conducted for the CIRCS from 1984 to 1992 in the Ikawa, Kyowa, and Noichi Districts, and from 1996 to 1998 in the Ozu Study. After exclusion of individuals with a history of stoke or coronary heart disease at baseline, 15,566 individuals were followed through the end of 2007 (192,181 person-years), and 263 ischemic strokes and 139 myocardial infarctions occurred among these populations. Of these, we selected 79 ischemic strokes and 52 myocardial infarctions in individuals who had fasting glucose levels ≥5.6 mmol/L or non-fasting glucose levels ≥7.2 mmol/L or who took medication for type 2 diabetes. In 117 (89.3%) of these cases, frozen blood samples at baseline were available for use in the present study. Controls were randomly selected at a 2:1 ratio among participants with available baseline blood samples and similar glucose levels, but without cardiovascular disease, and were matched for sex, age (±2 years), year of blood draw, fasting conditions, and community.

To identify strokes and coronary heart disease events, medical records were reviewed by trained nurses and physicians [20]. Incident strokes were validated based on the National Survey of Stroke criteria [21], which require a constellation of neurological deficits that present rapidly and persist for at least 24 h (or until death). For each subtype of stroke, i.e. subarachnoid hemorrhage, intracerebral hemorrhage, or ischemic stroke, a definitive diagnosis was established based on data from computer tomographic (CT) scans, magnetic resonance images (MRI), or autopsy.

Incident myocardial infarctions were confirmed in the medical records using the criteria of the MONICA project [22], which require electrocardiogram results, cardiac enzyme measurements, and/or autopsy. On the basis of the combined findings available for review, diagnoses of "definite myocardial infarction" and "possible myocardial infarction" were made. In the absence of a diagnosis of myocardial infarction, deaths that occurred within 1 h of onset were regarded as sudden cardiac deaths.

This study was approved by the Ethical Committees of Ehime University Graduate School of Medicine and University of Tsukuba.

2.2. Measurements

A self-administered questionnaire was completed by participants at baseline that included medical history, smoking and alcohol consumption habits, and time since the last meal. Blood pressure was measured on the right arm with subjects in a sitting position after resting at least 5 min by a trained technician using a standard mercury sphygmomanometer. Body mass index (BMI, kg/m [2]) was calculated as weight divided by height squared.

Subjects were considered to be fasting if blood was collected more than 8 h after the last meal. Total cholesterol and triglycerides were measured using conventional methods certified by the Osaka Medical Center for Health Science and Promotion, a member of the Cholesterol Reference Method Laboratory Network (CRMLN) [23]. Hypertension was defined as systolic and diastolic blood pressures ≥140/90 mmHg or the use of medication to treat hypertension. Hyperlipidemia was defined as total cholesterol ≥5.70 mmol/L, triglycerides ≥1.70 mmol/L, or the use of medication to treat dyslipidemia. Type 2 diabetes was defined as a fasting glucose level ≥7.0 mmol/L, a non-fasting glucose level ≥11.1 mmol/L, or the use of medication to treat diabetes.

Total and HMW adiponectin were measured using an enzyme-linked immunosorbent assay (ELISA) kit (SEKISUI Medical Co., LTD., Tokyo, Japan) [24]. The intra-assay coefficient of variation was 5.4% for total adiponectin (mean = 3.98 μg/mL) and 5.0% for HMW adiponectin (mean = 1.08 μg/mL). The lower limit for adiponectin detection was 0.038 ng/mL.

Covariates, including medical history, smoking status (current smoker, former smoker, or never smoked), and regular alcohol consumption (regular drinker, former drinker, or never drank) were determined using a self-administered questionnaire. Use of medications for hypertension or diabetes mellitus (yes/no) was determined by the question, "Have the following conditions been treated by physicians?" with hypertension, diabetes mellitus, and hyperlipidemia offered as potential responses.

2.3. Statistical analysis

With a mean of 12.3 years of follow-up time, person-years were calculated as the period from the date of baseline to that of the first endpoint (death, emigration, or loss) or until December 31, 2007.

Because of skewed distribution, total and HMW adiponectin values were log-transformed in analysis. When comparing means, medians, and percentages of variables between cases and controls, the unpaired t test, the Wilcoxon rank sum test, and the χ^2 test were used, respectively. Odds ratios and 95% confidence intervals (CIs) were calculated using a modified Poisson regression analysis adjusting for matched variables of sex, age (continuous), and community [25]. Furthermore, we constructed multivariable-adjusted models using smoking status (current smoker, others), alcohol consumption (regular alcohol drinker, others), and medications or past history of hypertension or diabetes (yes/no) as covariates. A test for linear trends was also performed using median values for total and HMW adiponectin grouped by quartile and adjusted for the same covariates. We examined interactions between adiponectin levels and subgroups with an interaction term (subgroup × adiponectin levels) using median values for each quartile in the multivariable-adjusted model.

Statistical significance was assumed at $P < 0.05$. All statistical analyses were performed using SAS software, version 9.2 (SAS Institute, Inc., Cary, North Carolina, USA).

3. Results

Table 1 compares established risk factors and adiponectin levels in cases and controls with high blood glucose levels. Body mass index was very similar between the groups. The prevalence of diabetes was significantly higher among cases than in controls. However, mean systolic and diastolic blood pressures and total cholesterol were not significantly different between groups. Furthermore, median levels of total adiponectin, HMW adiponectin, and HMW/total adiponectin ratios were not different between the groups.

Table 2 presents partial Spearman's correlation coefficients adjusted for sex, age, and community in control subjects. Total adiponectin, HMW adiponectin, and HMW/total adiponectin ratios were inversely associated with body mass index, triglycerides, and blood glucose. The correlation coefficients for total adiponectin, HMW adiponectin, and HMW/total adiponectin ratio ranged from 0.747 to 0.965. The partial Spearman's correlation coefficient between total and HMW adiponectin was 0.957 in case subjects.

Table 3 shows odds ratios for incident CVD among the second, third, and fourth quartiles of HMW adiponectin relative to the first quartile. Model 1 was adjusted for sex, age, and community. Model 2 was adjusted further for BMI, smoking, alcohol consumption, hyperlipidemia, hypertension, and type 2 diabetes. There was no significant association of total or HMW adiponectin with risk of CVD.

When stratified by age (40–69 years versus 70–85 years) (Table 4), the highest versus the lowest quartile of HMW adiponectin was significantly associated with CVD events in the younger age group (odds ratio = 0.33, 95% CI, 0.13–0.83, P for trend = 0.055). In addition, multivariable-adjusted models indicated that the association of the highest quartile for HMW/total adiponectin ratio with CVD events was also significant for individuals aged 40–69 years (odds ratio = 0.47, 95% CI, 0.22–0.98, P for trend = 0.047).

Table 1
Population characteristics among cases and controls.

	Total		Age groups			
			40–69 years		70–85 years	
	Case	Control	Case	Control	Case	Control
n	117	234	70	132	47	102
Men, %	69.2	69.2	74.3	73.5	61.7	63.7
Age, years	68.2	67.7	62.9	62.4	76.0	74.6
Body mass index, kg/m^2	23.8	23.8	24.3	24.1	23.0	23.5
Systolic blood pressure, mmHg	144.0	141.9	144.4	142.6	143.4	141.1
Diastolic blood pressure, mmHg	79.5	79.8	82.0	82.3	75.8	76.5
Total cholesterol, mmol/L	5.24	5.18	5.33	5.20	5.11	5.14
Triglycerides (median), mmol/L	1.54	1.39	1.79†	1.39	1.40	1.39
Glucose (median), mmol/L	8.60	8.21	8.71*	8.02	8.27	8.33
Hypertension, %	75.2	71.4	78.6	69.7	70.2	73.5
Diabetes, %	37.6‡	20.9	48.6‡	22.0	21.3	19.6
Dyslipidemia, %	69.2	62.0	74.3†	59.1	61.7	65.7
Current smoker, %	30.8	34.6	35.7	40.2	23.4	27.5
Current drinker, %	42.7*	52.4	42.9*	57.6	42.6	45.1
Total adiponectin (median), μg/mL	5.50	5.52	4.60	4.87	7.02	7.30
HMW adiponectin (median), μg/mL	2.67	2.73	1.92	2.27	3.74	3.79
HMW/total adiponectin ratio	0.48	0.50	0.45	0.47	0.53	0.51

*$P < 0.1$, †$P < 0.05$, ‡$P < 0.01$ versus controls tested in the conventional manner according to the distribution of each variable. HMW: high molecular weight.

Table 2
Partial Spearman's correlation coefficients adjusted for sex, age, and community in controls ($n = 234$).

	Total adiponectin		HMW adiponectin		HMW/total adiponectin ratio	
	r	P	r	P	r	P
Body mass index	−0.298	< 0.001	−0.293	< 0.001	−0.246	< 0.001
Systolic blood pressure	−0.017	0.795	−0.024	0.720	−0.044	0.505
Diastolic blood pressure	−0.023	0.725	−0.050	0.450	−0.100	0.131
Total cholesterol	0.042	0.527	0.018	0.792	−0.034	0.604
Triglycerides	−0.465	< 0.001	−0.439	< 0.001	−0.351	< 0.001
Glucose	−0.159	0.016	−0.136	0.039	−0.085	0.201
Total adiponectin	1.000		0.965	< 0.001	0.747	< 0.001
HMW adiponectin	0.965	<0.001	1.000		0.884	< 0.001
HMW/total adiponectin ratio	0.747	<0.001	0.884	< 0.001	1.000	

HMW: high molecular weight.

However, there was no association of any of the adiponectin markers with CVD events among individuals aged 70–85 years, although the odds ratios for the second, third, and fourth adiponectin quartiles in this age group tended to be higher than that for the lowest quartile. The interaction of the associations of age with HMW adiponectin and HMW/total adiponectin ratio was statistically significant.

4. Discussion

This prospective study found no overall association between total or HMW adiponectin concentrations and CVD events among individuals with high blood glucose levels. However, we found significant interactions between age groups for the associations of HMW adiponectin and HMW/total adiponectin ratio levels with CVD risk. In the 40–69 age group, the highest quartile for HMW adiponectin and HMW/total adiponectin ratio had a decreased risk of CVD events compared to the lowest quartile, but this association was not seen among the 70–85 age group. To our knowledge, this is the first prospective study to provide evidence that HMW adiponectin may be a predictor of CVD events among high-risk middle-aged individuals.

The Jichi Medical School (JMS) Cohort Study of Japan found no association between total adiponectin concentrations and CHD [26] or CVD events [27] in the general population. Overall weak associations were also reported in British [9,11] and U.S. populations [10,28], and in meta-analysis of these studies [8]; however, these studies did not measure HMW adiponectin or analyze the effects among age subgroups. In contrast, high HMW adiponectin levels have consistently been associated with a decreased risk of incident type 2 diabetes [6,29,30]. Indeed, adiponectin, and HMW adiponectin in particular, is thought to play an important role in antiatherogenic and anti-inflammatory activity and to enhance insulin sensitivity [31,32]. Therefore, an inverse association between adiponectin levels and risk of CVD might be expected given these properties of adiponectin [33]. Nonetheless, this inconsistency in the effects of adiponectin on incident CVD is not fully understood [5,34].

Adiponectin has a multimeric structure. Human adiponectin mutations that inhibit multimer formation lead to impaired glucose metabolism by abrogating the AMP-activated protein kinase pathway [2]. In addition, peroxisome proliferator-activated receptor (PPAR)-γ agonist treatment was shown to increase the HMW/total adiponectin ratio and to thereby improve hepatic insulin

Table 3
Sex-, age-, and community-adjusted and multivariable-adjusted odds ratios and 95% confidence intervals for cardiovascular disease shown by HMW adiponectin quartile.

	Quartile				Trend *P*
	Q1 (Low)	Q2	Q3	Q4 (High)	
Total adiponectin					
Median (range), μg/mL	3.06 (1.90–3.85)	4.60 (3.86–5.51)	7.30 (5.52–8.80)	10.87 (8.81–23.73)	
No. of cases/controls	32/58	27/59	33/58	25/59	
Model 1	1	0.85 (0.55–1.30)	0.96 (0.63–1.46)	0.77 (0.48–1.24)	0.39
Model 2	1	0.83 (0.55–1.26)	0.97 (0.64–1.48)	0.84 (0.51–1.38)	0.68
HMW adiponectin					
Median (range), μg/mL	1.04 (0.32–1.56)	2.03 (1.56–2.72)	3.67 (2.73–5.35)	6.75 (5.36–15.64)	
No. of cases/controls	30/58	30/59	36/58	21/59	
Model 1	1	0.96 (0.63–1.45)	1.06 (0.70–1.60)	0.70 (0.42–1.17)	0.15
Model 2	1	0.91 (0.60–1.37)	1.06 (0.70–1.61)	0.75 (0.44–1.28)	0.34
HMW/total adiponectin ratio					
Median (range)	0.32 (0.125–0.39)	0.43 (0.39–0.48)	0.53 (0.49–0.58)	0.64 (0.59–0.86)	
No. of cases/controls	32/58	32/59	25/58	28/59	
Model 1	1	0.98 (0.66–1.46)	0.81 (0.52–1.27)	0.85 (0.53–1.36)	0.39
Model 2	1	0.99 (0.67–1.48)	0.83 (0.53–1.28)	0.93 (0.57–1.51)	0.58

Model 1 was adjusted for sex, age, and community. Model 2 was adjusted further for body mass index, smoking, drinking, hyperlipidemia, hypertension, and type 2 diabetes. HMW: high molecular weight.

sensitivity [31,32]. Therefore, HMW adiponectin and HMW/total adiponectin ratio, but not total adiponectin, have been consistently associated with increased insulin sensitivity and vasculoprotective effects [35]. Since the present study showed that both HMW adiponectin and the HMW/total adiponectin ratio were associated with risk of CVD events, measurement of these multimeric forms of adiponectin may be especially useful for prediction of CVD risk. Regardless of the usefulness of HMW/total adiponectin ratio, it did not have the advantage of HMW adiponectin measurement in present study.

Of note, several prospective studies have shown that high adiponectin concentrations were not associated with decreased risk of CVD, but rather with a significantly elevated risk of CVD in elderly adults [12–14,36]. This phenomenon was in part explained by N-terminal pro-brain natriuretic peptide, a marker of cardiac dysfunction in the elderly, which is positively associated with adiponectin concentrations [37]. It is known that adiponectin levels increase with age. A decline in renal or cardiac function may be associated with the increased adiponectin levels seen in elderly people [14,38]. Furthermore, recent studies have revealed that adiponectin plays a role in preventing endothelial progenitor cell senescence by inhibiting the ROS/p38 MAP kinase/$p16^{INK4A}$ signaling cascade and contributes to endothelial repair in response to vascular damage [39]. These functions of adiponectin support the positive association between increased adiponectin levels and cardiac dysfunction or cardiovascular stress as indicated by increased NT-proBNP [5,14,37]. In the present study, moderate to high levels of HMW adiponectin in elderly adults tended to be associated with an increased risk of CVD, which may support the notion that elevated adiponectin is caused by cardiac dysfunction or other aging processes [34].

Additionally, we did not find the interaction association of adiponectin levels with sex. When stratified by outcomes, i.e. ischemic stroke versus myocardial infarction, multivariable-adjusted odds ratios of total and HMW adiponectin for ischemic stroke seemed to be decreased, whereas those for myocardial infarction was level or slightly increased. However, because of less myocardial infarction cases, it was not clear that the association of adiponectin levels was different between ischemic stroke and myocardial infarction cases.

Although we conducted a community-based prospective study with an extensive 12.3-year follow-up of 15,566 individuals and were able to examine different adiponectin isoforms, this study had several limitations. First, 75.5% of our blood samples were collected under non-fasting conditions, although adiponectin levels were not

Table 4
Multivariable-adjusted odds ratios and 95% confidence intervals for cardiovascular disease shown by HMW adiponectin quartile and stratified by age and body mass index.

Age group		Quartile				Trend *P*	Interaction *P*
		Q1 (Low)	Q2	Q3	Q4 (High)		
Total adiponectin							
40–69 years	No. of cases/controls	28/41	16/37	18/33	8/21		
	Multivariable-adjusted OR	1.00	0.58 (0.36–0.95)†	0.71 (0.43–1.17)	0.64 (0.30–1.34)	0.31	0.11
70–85 years	No. of cases/controls	4/17	11/22	15/25	17/38		
	Multivariable-adjusted OR	1.00	1.70 (0.59–4.83)	1.86 (0.67–5.18)	1.48 (0.53–4.11)	0.88	
HMW adiponectin							
40–69 years	No. of cases/controls	26/39	17/40	22/30	5/23		
	Multivariable-adjusted OR	1.00	0.54 (0.33–0.88)†	0.74 (0.45–1.21)	0.33 (0.13–0.83)†	0.055	0.047
70–85 years	No. of cases/controls	4/19	13/19	14/28	16/36		
	Multivariable-adjusted OR	1.00	2.49 (0.88–7.00)*	1.92 (0.68–5.46)	1.68 (0.61–4.68)	0.89	
HMW/total adiponectin ratio							
40–69 years	No. of cases/controls	25/34	20/43	15/27	10/28		
	Multivariable-adjusted OR	1.00	0.58 (0.37–0.92)†	0.66 (0.39–1.10)	0.47 (0.22–0.98)†	0.047	0.018
70–85 years	No. of cases/controls	7/24	12/16	10/31	18/31		
	Multivariable-adjusted OR	1.00	2.21 (0.98–5.02)*	1.16 (0.49–2.75)	1.71 (0.72–4.05)	0.57	

*$P < 0.1$, †$P < 0.05$. Odds ratios were adjusted for the same variables as model 2 in Table 3. HMW: high molecular weight; OR: odds ratio.