Table 4
Multivariate-adjusted hazard ratios for the development of ischemic stroke according to MetS statuses by various definitions by sex.

Criteria	Men			Women		
	Number of events/population at risk	Hazard ratio (95% confidence interval)	*P*	Number of events/population at risk	Hazard ratio (95% confidence interval)	*P*
Original Japanese						
MetS(−)	48/825	1.00		65/1289	1.00	
MetS(+)	18/225	1.32 (0.76–2.30)	0.33	14/113	2.09 (1.17–3.75)	0.01
Modified Japanese						
MetS(−)	51/945	1.00		48/1142	1.00	
MetS(+)	15/105	3.07 (1.68–5.61)	<0.001	31/260	2.21 (1.39–3.51)	<0.001
IDF						
MetS(−)	50/909	1.00		38/918	1.00	
MetS(+)	16/141	2.66 (1.47–4.81)	0.001	41/484	1.74 (1.11–2.73)	0.02
Original NCEP						
MetS(−)	54/874	1.00		49/1090	1.00	
MetS(+)	12/176	1.10 (0.58–2.07)	0.77	30/312	1.73 (1.09–2.76)	0.02
Modified NCEP						
MetS(−)	46/823	1.00		39/963	1.00	
MetS(+)	20/227	1.59 (0.93–2.74)	0.09	40/439	1.73 (1.10–2.71)	0.02

Adjusted for age, total cholesterol, proteinuria, electrocardiogram abnormalities, smoking habits, alcohol intake and regular exercise. MetS, Metabolic syndrome; IDF, International Diabetes Federation; NCEP, National Cholesterol Education Program.

4. Discussion

In a long-term prospective study of a general Japanese population, we demonstrated that MetS was an independent and significant risk factor for all of ischemic stroke subtypes when the modified Japanese criteria, in which a waist circumference of ≥90 cm in men and ≥80 cm in women was substituted for the original cutoff values, was used.

Several prospective studies [10–15] including ours [8], have investigated significant associations between MetS defined by the NCEP criteria or their modification and the risk of ischemic stroke. In these studies, however, ischemic stroke was not classified into clinical subtypes. Only a few studies have reported the relationship between MetS and ischemic stroke subtypes. In a hospital-based case–control study of elderly Greek subjects, the prevalence of MetS was higher in the non-embolic stroke group including LI and ATI than in the control group [18]. A case–control study for Japanese ischemic stroke patients [19] demonstrated that MetS was significantly related to ATI but not to LI and CEI. Another clinical study in Japan [20] revealed that the prevalence of MetS defined by the original Japanese criteria was highest among patients with CEI followed by those with LI. To our knowledge, the present study is the first population-based prospective cohort study to investigate the association between MetS and the development of each ischemic stroke subtype.

Among the several MetS criteria, the cutoff values of waist circumference to define abdominal obesity are largely different. Because the cutoff values of waist circumference in the original NCEP criteria (>102 cm in men and >88 cm in women) were created for American subjects [5], these values seem to be unsuitable for the Japanese population. The original Japanese criteria used the cutoff values of ≥85 cm in men and ≥90 cm in women based on correlations with visceral fat mass [7]. However, the IDF has claimed that using these values produces "odd results" in relation to cardiovascular risk and recommends the use of cutoff values of ≥90 cm in men and ≥80 cm in women for Asian populations including Japanese [6]. In our previous study, we compared the ability to predict cardiovascular disease at each published cutoff level of waist circumference among the MetS criteria and demonstrated that the optimal cutoff point of waist circumference was 90 cm in men and 80 cm in women [9]. In the present study, we observed a similar result for the risk of ischemic stroke in men (Table 3). Therefore, we created the modified Japanese and the modified NCEP criteria, which substitute waist circumference cutoff values of ≥90 cm in men and ≥80 cm in women for the original values. Among these five criteria, we found that the modified Japanese criteria were the best at predicting the risk of ischemic stroke and its subtypes. These findings are concordant with those of our previous study [9], in which MetS defined by the modified Japanese criteria was a better predictor for the development of cardiovascular disease.

In this study, the risks of ischemic stroke and all subtypes were higher for the modified Japanese MetS criteria than for the IDF or the modified NCEP criteria despite the identical cutoff values of waist circumference. One reason for this is the difference in the definition of hyperglycemia: the definition of hyperglycemia in the modified Japanese criteria (≥6.1 mmol/L) was superior to that in the IDF criteria (≥5.6 mmol/L) for the prediction of ischemic stroke in our subjects (Table 3). Another reason seems to be that abdominal obesity is an essential component for the modified Japanese criteria, but not for the NCEP criteria. These findings support the opinion that abdominal obesity should be an essential component for the diagnosis of MetS though there has been controversy over the necessity of abdominal obesity.

Our study demonstrated that MetS defined by the modified Japanese criteria appears to be a significant risk factor for the development of LI. Very few studies have examined the relationship between MetS and LI. A cross-sectional study recently demonstrated a significant association between MetS and silent LI [21]. LI develops due mainly to arteriolosclerosis such as lipohyalinosis, fibrinoid necrosis or microatheroma in penetrating arteries of the brain [22]. Some disorders in secretion of adipocytokines have been observed in the MetS status. For example, it was reported that plasma concentrations of adiponectin decreased in subjects with abdominal obesity [23], and lower adiponection levels were associated with impaired endothelial function [24]. It has also been demonstrated that the plasma concentration of plasminogen activator inhibitor-1 (PAI-1) increased in subjects with abdominal obesity [25], and overexpression of PAI-1 was associated with subendocardial myocardial infarction as a result of perivascular fibrosis and thrombosis in penetrating coronary arteries in PAI-1 transgenic mice [26]. It is reasonably considered that similar arteriosclerotic lesions may also occur in penetrating brain arteries. Therefore, adipocytokine disorders may be related to endothelial

Table 5
Multivariate-adjusted hazard ratios for the development of ischemic stroke subtypes according to MetS status by various definitions.

Criteria	Lacunar infarction			Atherothrombotic infarction			Cardioembolic infarction		
	Number of events/population at risk	Hazard ratio (95% confidence interval)	P	Number of events/population at risk	Hazard ratio (95% confidence interval)	P	Number of events/population at risk	Hazard ratio (95% confidence interval)	P
Original Japanese									
MetS(−)	57/2114	1.00		31/2114	1.00		25/2114	1.00	
MetS(+)	15/338	1.50 (0.82–2.72)	0.19	9/338	1.61 (0.76–3.43)	0.22	8/338	1.96 (0.87–4.45)	0.11
Modified Japanese									
MetS(−)	51/2,087	1.00		28/2087	1.00		20/2087	1.00	
MetS(+)	21/365	1.94 (1.13–3.32)	0.02	12/365	2.55 (1.25–5.18)	0.01	13/365	3.94 (1.89–8.22)	<0.001
IDF									
MetS(−)	44/1,827	1.00		25/1827	1.00		19/1827	1.00	
MetS(+)	28/625	1.65 (0.98–2.78)	0.06	15/625	2.15 (1.06–4.34)	0.03	14/625	2.69 (1.27–5.68)	0.01
Original NCEP									
MetS(−)	50/1964	1.00		29/1964	1.00		24/1964	1.00	
MetS(+)	22/488	1.48 (0.88–2.47)	0.14	11/488	1.37 (0.67–2.79)	0.38	9/488	1.47 (0.67–3.21)	0.34
Modified NCEP									
MetS(−)	44/1,786	1.00		23/1786	1.00		18/1786	1.00	
MetS(+)	28/666	1.35 (0.83–2.22)	0.23	17/666	1.90 (0.99–3.63)	0.05	15/666	2.20 (1.08–4.45)	0.03

Adjusted for age, total cholesterol, proteinuria, electrocardiogram abnormalities, smoking habits, alcohol intake and regular exercise. MetS, Metabolic syndrome; IDF, International Diabetes Federation; NCEP, National Cholesterol Education Program.

dysfunction and induce arteriolosclerotic lesions in the brain leading to the development of LI.

In our subjects, MetS defined by the modified Japanese or IDF criteria was also clearly associated with the occurrence of ATI. ATI is caused by atherosclerosis in extracranial or intracranial arteries. There have been several other studies demonstrating associations between MetS and atherosclerotic lesions in extracranial or intracranial arteries [27,28].

In this study, MetS defined by the modified Japanese, IDF or modified NCEP criteria was associated with the development of CEI. CEI occurs due to thromboembolism from the heart to the arteries of the brain as a result of cardiac diseases such as atrial fibrillation, valvular heart diseases and myocardial infarction [17]. It was recently shown in a cohort study that MetS was a significant risk factor for the development of atrial fibrillation [29], which is the most common embolic source of CEI. In our study, the prevalence of atrial fibrillation at baseline was significantly higher in the subjects with CEI than in those without CEI (21.2% vs. 0.9%, $P<0.001$). Consequently, it is considered that atrial fibrillation occurs on the pathway between MetS and CEI.

The strengths of our study include accurate measurement of MetS components including waist circumference at baseline, longitudinal population-based study design, long duration of follow-up, perfect follow-up of subjects and accuracy for diagnosis of stroke including ischemic stroke subtypes. One limitation of our study is that the diagnosis of MetS and other risk factors was based on a single measurement at baseline, as has been the case in other epidemiological studies. During the follow-up, risk factor levels could be changed due to modifications in lifestyle or medication; hence, misclassification of MetS is possible. This would weaken the association found in this study, biasing the results toward the null hypothesis. Therefore, the true association may be stronger than that shown in our study.

In conclusion, we have shown that MetS defined by the modified Japanese criteria is an independent and significant risk factor for the development of all ischemic stroke subtypes. In these criteria, the impact of MetS on the occurrence of CEI was largest, followed by those of ATI and LI. Because the prevalence of metabolic disorders has shown a steep increase during the past several decades in the overall Japanese population [3], our findings indicate that correction of MetS is important for prevention of all ischemic stroke subtypes in Japan.

Conflict of interest

No authors have any conflict of interest.

Acknowledgements

The authors thank the staff of the Division of Health and Welfare of Hisayama for their cooperation in this study. This study was supported in part by a Grant-in-Aid for Scientific Research A (No. 18209024) from the Ministry of Education, Culture, Sports and Technology of Japan.

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Nephrol Dial Transplant (2010) 25: 3236–3240
doi: 10.1093/ndt/gfq155
Advance Access publication 25 March 2010

Circulating resistin is increased with decreasing renal function in a general Japanese population: the Hisayama Study

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Abstract

Background. The purpose of this study is to investigate the relationship between serum resistin levels and chronic kidney disease (CKD).

Methods. A total of 3192 community-dwelling subjects (1377 men, 1815 women), aged ≥40 years and without renal failure, were divided into four groups according to quartiles of serum resistin concentrations: ≤7.1, 7.2–9.9, 10.0–14.7 and ≥14.8 ng/mL. The associations of resistin levels with renal function status were examined cross-sectionally. The estimated glomerular filtration rate (eGFR) was calculated using the equation from the Modification of Diet in Renal Disease Study, and CKD was defined as an eGFR of <60 mL/min/1.73 m^2.

Results. The age- and sex-adjusted mean values of eGFR decreased significantly with elevating quartiles of resistin (P for trend <0.001). The age- and sex-adjusted odds ratios (ORs) for the presence of CKD increased progressively with higher quartiles of resistin. This trend remained robust even after controlling for age, sex, body mass index, diabetes, homeostasis model assessment of insulin resistance (HOMA-IR), high-sensitivity C-reactive protein (hs-CRP), triglycerides, high-density lipoprotein and total cholesterol, hypertension, current smoking, current drinking, and regular exercise [second quartile: OR 1.44, 95% confidence interval (CI) 1.05–1.99; third quartile: OR 2.15, 95% CI 1.58–2.92; fourth quartile: OR 2.32, 95% CI 1.71–3.16; P for trend <0.001]. In stratified analyses, high resistin level (≥7.2 ng/mL) was a significant relevant factor in CKD, independent of HOMA-IR or hs-CRP level.

Conclusion. Our findings suggest that elevated resistin level is significantly associated with the likelihood of CKD in the general Japanese population.

Keywords: chronic kidney disease; cross-sectional study; epidemiology; resistin

Introduction

Chronic kidney disease (CKD) is a worldwide public health concern and a major risk factor for end-stage renal disease, cardiovascular disease and premature death [1]. Identifying and treating risk factors for mild CKD may be the best approach to prevent and delay advanced outcomes [1]. Several epidemiological studies associated age, high blood pressure, diabetes, proteinuria, dyslipidaemia and smoking with the subsequent decline in estimated glomerular filtration rate (eGFR) [2,3]. It was also reported that insulin resistance and inflammation were emerging risk factors for the occurrence of CKD [4,5]. However, regardless of the treatment and prevention of these factors, patients with renal failure are increasing in number [6], suggesting the presence of other risk factors.

Resistin belongs to a family of cysteine-rich secretory proteins called resistin-like molecules [7]. In rodents, resistin is derived almost exclusively from fat tissue, and its serum levels are elevated in animal models of obesity and insulin resistance [8]. In humans, on the other hand, resistin is highly expressed in monocytes and macrophages [9]; thus, its pathophysiological role may differ between species. *In vitro*, resistin activated human endothelial cells, leading to increased expression of adhesion molecules, and induced human aortic muscle cell proliferation [10]. Furthermore, some clinical and epidemiological studies revealed positive correlations between plasma resistin levels and pro-inflammatory cytokines [11,12]. A few recent clinical studies also showed an inverse correlation between resistin level and eGFR in CKD patients [13–15]. To date, however, there have been no investigations into the link between serum resistin levels and CKD in large general populations excluding patients with renal failure. The aim of the present study is to examine the relationship between serum resistin levels and CKD in a cross-sectional study of a general Japanese population.

Table 1. Age- and sex-adjusted mean values or frequencies of risk factors according to quartiles of serum resistin concentrations

Variable	Serum resistin levels (ng/mL)				P-value for trend
	1.5–7.1 (n = 788)	7.2–9.9 (n = 803)	10.0–14.7 (n = 805)	14.8–90.2 (n = 796)	
Age (years)	59 ± 11	61 ± 12	62 ± 13	64 ± 13	<0.001
Men (%)	38.1	40.4	44.6	49.5	<0.001
BMI (kg/m^2)	23.0 ± 3.4	23.1 ± 3.4	23.0 ± 3.4	23.2 ± 3.4	0.50
Serum creatinine (μmol/L)	59 ± 15	62 ± 15	64 ± 15	65 ± 15	<0.001
Fasting plasma glucose (mmol/L)	6.1 ± 1.3	6.1 ± 1.3	6.0 ± 1.3	6.1 ± 1.3	0.70
Fasting insulin (pmol/L)	44.6 (13.8–144.5)	46.6 (14.5–150.0)	46.3 (14.4–148.7)	49.5 (15.3–160.5)	0.001
Diabetes (%)	16.5	16.8	18.0	18.9	0.45
HOMA-IR	1.66 (0.44–6.29)	1.72 (0.46–6.48)	1.71 (0.45–6.42)	1.83 (0.48–6.94)	0.007
Hs-CRP (mg/L)	0.44 (0.04–4.53)	0.51 (0.05–5.28)	0.54 (0.05–5.49)	0.66 (0.06–6.88)	<0.001
Triglycerides (mmol/L)	1.12 (0.39–3.18)	1.16 (0.41–3.29)	1.14 (0.40–3.23)	1.17 (0.41–3.35)	0.13
HDL-cholesterol (mmol/L)	1.70 ± 0.40	1.61 ± 0.40	1.62 ± 0.40	1.55 ± 0.40	<0.001
Total cholesterol (mmol/L)	5.30 ± 0.90	5.27 ± 0.89	5.31 ± 0.89	5.23 ± 0.90	0.15
Systolic blood pressure (mmHg)	132 ± 20	132 ± 20	132 ± 20	132 ± 20	0.96
Diastolic blood pressure (mmHg)	79 ± 12	79 ± 12	78 ± 12	78 ± 12	0.66
Hypertension (%)	45.0	42.9	43.2	46.3	0.98
Current smoking (%)	20.7	21.5	21.9	22.8	0.29
Current drinking (%)	48.8	44.4	43.4	38.3	<0.001
Regular exercise (%)	11.1	10.4	9.8	8.7	0.04

Values are given as means ± SD or frequencies. Age and percentage of men are not adjusted. Fasting insulin, HOMA-IR, hs-CRP and triglycerides are shown by geometric means and 95% CIs due to the skewed distribution.BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein.

Materials and methods

Study population

A population-based prospective study of cardiovascular disease has been under way since 1961 in the town of Hisayama, a suburb of Fukuoka City in southern Japan. As part of this study, in 2002, we conducted a cross-sectional examination among residents of the town. A detailed description of this survey was published previously [16]. Briefly, of all residents aged 40 years or over, 3328 underwent the examination (participation rate, 77.6%). After excluding 30 subjects who did not consent to participate in the study, 13 subjects with renal failure (eGFR <15 mL/min/1.73 m^2 or treated by dialysis), 82 subjects who had already eaten breakfast on the day serum samples were to be taken and 11 subjects without serum samples for resistin measurement, the remaining 3192 subjects (1377 men, 1815 women) were enrolled in this study.

This study was conducted with the approval of the Ethics Committee of the Faculty of Medicine, Kyushu University. Written informed consent was obtained from all participants.

Clinical evaluation and laboratory measurements

At the screening examination, blood samples were collected from an antecubital vein between 8:00 and 10:30 a.m. after at least a 12-h overnight fast. A portion of each serum specimen was stored at −80°C for 5 years, until 2007, when it was used for the measurement of resistin concentrations by a human resistin enzyme-linked immunosorbent assay kit supplied by R&D Systems (Minneapolis, MN) following the manufacturer's protocol. Linearity was maintained <0.16 ng/mL, and both intra- and inter-assay coefficient variations were comparable to those specified by the manufacturer (2.6–10.5%). Using fresh blood samples, serum creatinine was measured by the enzymatic method. Levels of triglycerides, high-density lipoprotein (HDL) and total cholesterol were determined enzymatically. Blood for the glucose assay was obtained by venipuncture into tubes containing sodium fluoride, and plasma glucose concentrations were determined by the glucose oxidase method. Subjects were considered to have diabetes mellitus if they had a fasting plasma glucose level of ≥7.0 mmol/L, had a 2-h post-load glucose level of ≥11.1 mmol/L or were taking antidiabetic medications. Serum insulin values were measured by a chemiluminescent enzyme immunoassay. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated with the formula fasting plasma glucose (mmol/L) × fasting serum insulin (μU/mL) / 22.5 [17], and subjects in the top quartile of HOMA-IR distribution were defined as having insulin resistance [18]. High-sensitivity C-reactive protein (hs-CRP) levels were quantified using a modification of the Behring latex-enhanced CRP assay on a Behring nephelometer BN-100 (Behring Diagnostics, Westwood, MA). High CRP values were defined as ≥1.0 mg/L, according to our previous report [19].

Blood pressure was obtained three times using an automated sphygmomanometer (BP-203RV III; Colin, Tokyo, Japan) with the subjects in a sitting position; the average of the three measurements was used in the present analysis. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or current treatment with antihypertensive agents. Height and weight were measured with the subject wearing light clothes without shoes, and body mass index [BMI (kg/m^2)] was calculated.

Each participant completed a self-administered questionnaire covering medical history, smoking habit, alcohol intake and exercise. The questionnaire was checked by trained interviewers at the screening. Smoking habit and alcohol intake were classified as either current habitual use or not. Those subjects who engaged in sports or other forms of exertion ≥3 times a week during their leisure time made up a regular exercise group.

Definition of CKD

GFR was estimated by using the following modified equation of the Modification of Diet in Renal Disease (MDRD) Study for Japanese [20]: eGFR (mL/min/1.73 m^2) = 175 × [serum creatinine (mg/dL)]$^{-1.154}$ × [age (years)]$^{-0.203}$ × [0.741 (Japanese coefficient)] × (0.742 if female). Based on the National Kidney Foundation Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines [1], we divided kidney function levels into four categories according to eGFR: normal and CKD stage 1 (eGFR ≥90 mL/min/1.73 m^2), CKD stage 2 (eGFR 60–89 mL/min/1.73 m^2), CKD stage 3 (eGFR 30–59 mL/min/1.73 m^2) and CKD stage 4 (eGFR 15–29 mL/min/1.73 m^2). We also determined CKD as a dichotomized category when eGFR was <60 mL/min/1.73 m^2.

Statistical analysis

SAS software package version 8.2 (SAS Institute, Cary, NC) was used to perform all statistical analyses. Because the distributions of fasting insulin, HOMA-IR, hs-CRP and triglycerides were skewed, these variables were natural log-transformed for statistical analysis. The subjects were divided into quartiles of resistin concentrations: ≤7.1, 7.2–9.9, 10.0–14.7 and ≥14.8 ng/mL. The mean values of possible risk factors were adjusted for

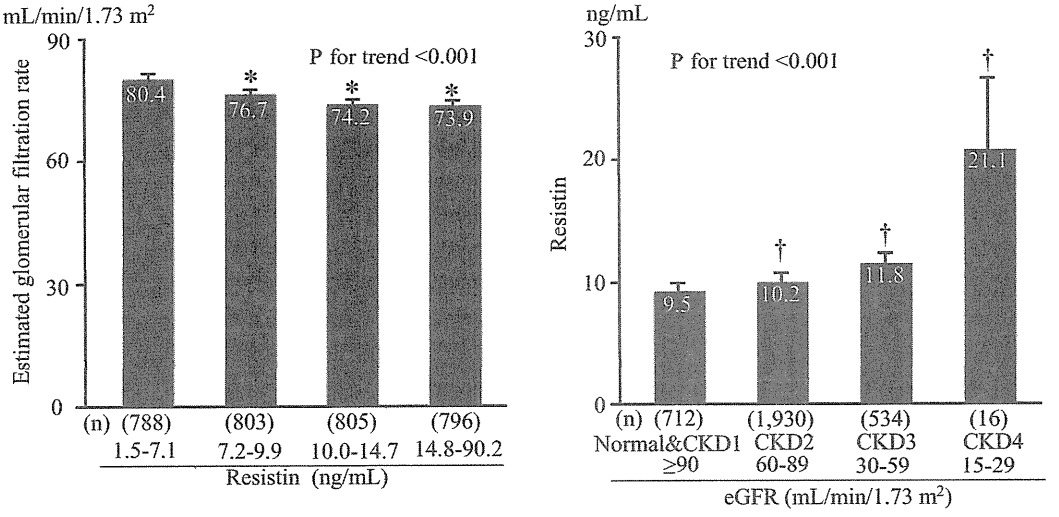

Fig. 1. The age- and sex-adjusted mean values of estimated glomerular filtration rate (eGFR) according to quartiles of serum resistin concentrations (left panel), and the age- and sex-adjusted mean values of serum resistin levels according to eGFR (right panel). Values are given as means ± standard error. *P < 0.001 *vs* the first quartile, †P < 0.001 *vs* eGFR of ≥90 mL/min/1.73 m^2.

age and sex using the analysis of covariance and were compared among the resistin quartiles according to the linear regression model. Age- and sex-adjusted means of eGFR among the quartiles were determined by the same method. The frequencies of risk factors were adjusted for age and sex by the direct method using all subjects as a standard population and were tested for trends using logistic regression analysis. The age- and sex- or multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for CKD were also determined by logistic regression analysis.

Results

Table 1 shows the age- and sex-adjusted means or frequencies of potential risk factors according to the quartiles of serum resistin concentrations. The mean values of age, serum creatinine, fasting insulin, HOMA-IR and hs-CRP, as well as the percentage of men, increased with the quartiles of resistin values, while the mean HDL cholesterol concentration and the frequencies of alcohol intake and regular exercise were negatively correlated with the quartiles. The other variables were not significantly associated with the quartiles.

The left panel in Figure 1 shows the age- and sex-adjusted mean values of eGFR according to the quartiles of serum resistin values. The mean values of eGFR decreased significantly with the quartiles of resistin values (80.4, 76.7, 74.2 and 73.9 mL/min/1.73 m^2, respectively; P for trend <0.001). Meanwhile, the age- and sex-adjusted mean values of serum resistin according to eGFR are shown in the right panel in Figure 1. The age- and sex-adjusted geometric mean values of serum resistin increased significantly as eGFR decreased (9.5 ng/mL in the normal and CKD stage 1, 10.2 ng/mL in CKD stage 2, 11.8 ng/mL in CKD stage 3 and 21.1 ng/mL in CKD stage 4; P for trend <0.001): the differences were significant between normal and CKD stage 1 and CKD stages 2–4 (all P < 0.001).

Table 2 shows the age- and sex-adjusted or multivariate-adjusted ORs and 95% CIs for the presence of CKD according to the resistin quartiles. The age- and sex-adjusted OR for CKD significantly increased with elevating quartiles (P for trend <0.001); compared to the first quartile, OR was greater in the second to fourth quartiles. Such associations were substantially unchanged after adjustment for age, sex, BMI, diabetes, HOMA-IR, hs-CRP, triglycerides, HDL and total cholesterol, hypertension, current smoking, current drinking, and regular exercise (second quartile: OR 1.44, 95% CI 1.05–1.99; third quartile: OR 2.15, 95% CI 1.58–2.92; fourth quartile: OR 2.32, 95% CI 1.71–3.16).

Table 2. Age- and sex- or multivariate-adjusted ORs and their 95% CIs for the presence of chronic kidney disease according to quartiles of serum resistin concentrations

	Serum resistin levels (ng/mL)				
	1.5–7.1	7.2–9.9	10.0–14.7	14.8–90.2	P-value for trend
Subjects (*n*)	788	803	805	796	
CKD cases (*n*)	75	117	166	192	
Age- and sex-adjusted OR (95% CI)	1 (reference)	1.44 (1.06–1.98)	2.15 (1.59–2.90)	2.33 (1.73–3.14)	<0.001
Multivariate-adjusted OR (95% CI)	1 (reference)	1.44 (1.05–1.99)	2.15 (1.58–2.92)	2.32 (1.71–3.16)	<0.001

Multivariate adjustment was made for age, sex, body mass index, diabetes, homeostasis model assessment of insulin resistance, high-sensitivity C-reactive protein, triglycerides, high-density lipoprotein and total cholesterol, hypertension, current smoking, current drinking, and regular exercise.CKD, chronic kidney disease; OR, odds ratio; CI, confidence interval.

Table 3. Age- and sex- or multivariate-adjusted ORs and their 95% CIs for chronic kidney disease according to the presence or absence of high resistin levels and high HOMA-IR as well as high hs-CRP values

	Subjects, *n*	CKD cases, *n*	Age- and sex-adjusted OR (95% CI)	P-value	Multivariate-adjusted OR (95% CI)	P-value
HOMA-IR						
Low + low resistin	602	57	1 (reference)		1 (reference)	
Low + high resistin	1773	340	1.84 (1.35–2.49)	<0.001	1.85 (1.35–2.52)	<0.001
High + low resistin	178	17	1.00 (0.56–1.78)	0.99	0.93 (0.51–1.68)	0.80
High + high resistin	613	131	2.34 (1.66–3.29)	<0.001	2.14 (1.47–3.12)	<0.001
hs-CRP						
Low + low resistin	645	53	1 (reference)		1 (reference)	
Low + high resistin	1717	309	2.13 (1.56–2.91)	<0.001	2.12 (1.54–2.91)	<0.001
High + low resistin	143	22	1.69 (0.98–2.92)	0.06	1.62 (0.92–2.84)	0.09
High + high resistin	687	166	2.45 (1.74–3.45)	<0.001	2.46 (1.72–3.50)	<0.001

High resistin levels were defined as the second or higher quartiles of its values; low resistin levels were the first quartile of its values.HOMA-IR: 'high' indicates ≥75th percentile (HOMA-IR ≥2.6); 'low' <75th percentile.Hs-CRP: 'high' indicates ≥1.0 mg/L; 'low' <1.0 mg/L. Multivariate adjustment was made for age, sex, body mass index, diabetes, HOMA-IR, hs-CRP, triglycerides, high-density lipoprotein and total cholesterol, hypertension, current smoking, current drinking, and regular exercise, but each risk factor that had been used for categorization was excluded from the confounding factors.CKD, chronic kidney disease; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; CI, confidence interval.

Finally, we examined the combined as well as separate effects of resistin and HOMA-IR or hs-CRP levels on the presence of CKD (Table 3). When a high resistin level was in the second or higher quartile (≥7.2 ng/mL), the age- and sex-adjusted ORs of CKD were significantly higher in subjects with high resistin and low HOMA-IR (<75th percentile) and in subjects with high resistin and high HOMA-IR (≥75th percentile) compared to the reference group, who had low resistin and low HOMA-IR. Similarly, the age- and sex-adjusted risks of CKD were significantly higher in subjects with high resistin levels, independent of hs-CRP levels. These relationships remained robust even after adjusting for the confounding factors named above.

Discussion

Using the large cross-sectional data of a general Japanese population, we demonstrated that serum resistin levels were negatively associated with eGFR, and that the mean values of serum resistin increased even in CKD stage 2. Furthermore, the elevated levels of serum resistin were an independent relevant factor for CKD after controlling for age, sex, BMI, diabetes, HOMA-IR, hs-CRP, triglycerides, HDL and total cholesterol, hypertension, current smoking, current drinking, and regular exercise. In stratified analyses, high resistin levels were associated significantly with the likelihood of CKD in those with low HOMA-IR (<75th percentile) as well as in those with low hs-CRP levels (<1.0 mg/L). These findings suggest that the measurement of resistin values provides additional information on the risk factors for CKD.

In a few clinical studies of CKD patients, resistin levels have been shown to be inversely correlated with eGFR [13–15]. Although polypeptides that have molecular weights comparable to those of resistin are thought to be freely filtered at the normal glomerulus [21], subjects with advanced renal impairment might have serum resistin accumulations due to reduced renal clearance: that is, renal dysfunction might cause elevated serum resistin levels. However, the present study showed that resistin levels were significantly raised even in subjects with CKD stage 2 (eGFR of 60–89 mL/min/1.73 m^2), in which polypeptides would be filtered almost normally. In a clinical study of patients with immunoglobulin A glomerulonephritis, serum resistin levels were also significantly higher in subjects with mild renal dysfunction who had a mean GFR of 76 mL/min/1.73 m^2 than in those who had a mean GFR of 114 mL/min/1.73 m^2 [13]. These observations support the hypothesis that resistin potentially plays an important role in the development of CKD.

It has been assumed that the effect of resistin is mediated via insulin resistance or inflammation. In our study, serum resistin levels were positively associated with HOMA-IR, but the association between circulating resistin levels and the likelihood of CKD was independent of HOMA-IR, which is in accord with the results of other clinical studies [13,14,22]. Thus, insulin resistance may not be a major mediator of the association between resistin levels and the risk of CKD. Meanwhile, the present study also indicated that the association between resistin levels and the likelihood of CKD was unrelated to hs-CRP, though resistin levels were closely associated with those levels. Resistin directly stimulated the expression of pro-inflammatory cytokines such as tumour necrosis factor-α and interleukin-6 in human peripheral blood mononuclear cells [23], and increased the expression of vascular cell adhesion molecule-1 and intracellular adhesion molecule-1 genes in vascular endothelial cells [10]. Furthermore, a recent clinical study provided evidence that the association between plasma resistin levels and plasma monocyte chemoattractant protein-1 concentrations was independent of hs-CRP levels [24]. Since it has become apparent that CKD is a state of chronic glomerular inflammation [5], resistin may play an important role in potentiating inflammation in the kidney, irrespective of hs-CRP and other confounding factors.

There are a variety of methods for estimating GFR. Although inulin clearance is the gold standard among these

methods, it is difficult to perform in routine practice. During the past decade, a great deal of effort has been devoted to establishing an equation for estimating GFR. There is a debate about which equation is optimal for Japanese; the original MDRD Study equation is widely accepted by clinical practitioners, but it may be unsuitable for Asian populations [20]. When some existing equations, such as the original MDRD Study equations [25] or the Cockcroft–Gault formula [26], were used instead of the modified MDRD Study equation for Japanese in our subjects, we also found significant associations between elevated resistin levels and CKD (data not shown). These findings imply a robust association between serum resistin levels and CKD.

A limitation of our study should be discussed. Due to the study's cross-sectional design, we cannot exclude the possibility that hyperresistinaemia is a consequence of CKD, which is a condition of low urine excretion from serum. However, we found significantly increased resistin levels even when eGFR was 60–89 mL/min/1.73 m^2. Thus, we believe that elevated resistin levels are a potential risk factor for the development of CKD.

In conclusion, an elevated resistin level was a significant relevant factor for CKD in a general Japanese population after taking into account other risk factors, including HOMA-IR and hs-CRP. Because of the cross-sectional design of this study, it is still unclear whether or not hyperresistinaemia is a cause of CKD. Further prospective studies are needed to clarify the causative relationship between serum resistin concentrations and CKD.

Acknowledgements. This study was supported in part by Grants-in-Aid for Scientific Research A (no. 18209024) and C (no. 20591063) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a Health and Labour Sciences Research Grant of the Ministry of Health, Labour and Welfare of Japan (Comprehensive Research on Aging and Health: H20-Chouju-004). The authors thank the staff of the Division of Health and Welfare of Hisayama for their cooperation in this study.

Conflict of interest statement. None declared.

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Received for publication: 28.9.09; Accepted in revised form: 25.2.10

Insulin resistance is associated with the pathology of Alzheimer disease

The Hisayama Study

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ABSTRACT

Objective: We examined the association between diabetes-related factors and pathology of Alzheimer disease (AD) to evaluate how diabetes affects the pathogenic process of AD.

Methods: This study included specimens from a series of 135 autopsies of residents of the town of Hisayama in Fukuoka prefecture (74 men and 61 women) performed between 1998 and 2003, who underwent a 75-g oral glucose tolerance test in clinical examinations in 1988. We measured diabetes-related factors including fasting glucose, 2-hour post-load plasma glucose, fasting insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) in 1988. Neuritic plaques (NPs) were assessed according to the Consortium to Establish a Registry for Alzheimer's Disease guidelines and neurofibrillary tangles (NFTs) were assessed according to Braak stage. The associations between each factor and AD pathology were examined by analysis of covariance and logistic regression analyses.

Results: Higher levels of 2-hour post-load plasma glucose, fasting insulin, and HOMA-IR were associated with increased risk for NPs after adjustment for age, sex, systolic blood pressure, total cholesterol, body mass index, habitual smoking, regular exercise, and cerebrovascular disease. However, there were no relationships between diabetes-related factors and NFTs. Regarding the effects of *APOE* genotype on the risk of AD pathology, the coexistence of hyperglycemia and *APOE* ϵ4 increased the risk for NP formation. A similar enhancement was observed for hyperinsulinemia and high HOMA-IR.

Conclusion: The results of this study suggest that hyperinsulinemia and hyperglycemia caused by insulin resistance accelerate NP formation in combination with the effects of *APOE* ϵ4.
Neurology® 2010;75:764–770

GLOSSARY

AD = Alzheimer disease; **BMI** = body mass index; **CERAD** = Consortium to Establish a Registry for Alzheimer's Disease; **CI** = confidence interval; **FPG** = fasting plasma glucose; **GSK3** = glycogen synthase kinase 3; **HOMA-IR** = homeostasis model assessment of insulin resistance; **IDE** = insulin-degrading enzyme; **NFT** = neurofibrillary tangle; **NP** = neuritic plaque; **OGTT** = oral glucose tolerance test; **OR** = odds ratio; **PG** = post-load plasma glucose.

The prevalence of diabetes is growing at epidemic proportions worldwide, and is becoming a major health problem. Several large longitudinal population-based studies have shown that the rate of cognitive decline is accelerated in elderly people with type 2 diabetes compared with the general population.[1-3] Similarly, other epidemiologic studies have revealed that diabetes increases the risk of dementia,[2,4-7] including Alzheimer disease (AD), which is the most common cause of dementia in late life.[2,4,5,8,9] Therefore, the effect of diabetes on cognitive function in the elderly has significant public health implications.

Several lines of evidence indicate a role of insulin and glucose metabolism on the risk of developing dementia, including AD.[10-14] Many mechanisms through which diabetes could increase the risk of dementia have been postulated, and include glucose toxicity, insulin resis-

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e-Pub ahead of print on August 25, 2010, at www.neurology.org.

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Study funding: Supported by a Grant-in-Aid for Scientists from the Ministry of Health, Labour and Welfare, Japan (H19-nanchi-ippan-006), a Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare (Comprehensive Research on Aging and Health: H20-Choujuu-004), and a Grant-in-Aid for Scientists from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 19300125).

Disclosure: Author disclosures are provided at the end of the article.

tance, oxidative stress, advanced glycation end products, inflammatory cytokines, and microvascular and macrovascular disease.[15] However, the determinant pathway, which is more critical to AD pathogenesis, is less clear. Understanding the role of disease-related risk factors for AD pathogenesis may help to identify specific modifiable risk factors that could enable the prevention of AD.[16] Therefore, identifying the dominant pathway through which diabetes influences the pathogenic process of AD may have benefits for public health.

To clarify the relationship between diabetes and AD, we searched for evidence of AD-related pathologic risk by examining the associations between diabetes-related factors and typical AD-related pathologic outcomes, neuritic plaques (NPs) and neurofibrillary tangles (NFTs).

METHODS **Subjects.** Since 1961, we have been conducting a long-term prospective cohort study of cerebro-cardiovascular diseases in the town of Hisayama, a suburb of the city of Fukuoka in southern Japan. The design of the Hisayama Study has been described in detail elsewhere.[17-19] In the present study, we examined a series of autopsy samples of Hisayama residents from October 1, 1998, to March 31, 2003. During this period, 290 residents in Hisayama died and 214 were autopsied (autopsy rate: 73.8%). The clinical data for the present study were collected from a clinical examination performed in 1988, as described previously.[19] Briefly, of a total of 3,390 residents aged over 40 years included in this registry, 2,742 (participation rate, 80.9%) took part in a clinical examination in 1988. Of these, a 75-g oral glucose tolerance test (OGTT) was performed in 2,520 subjects. Of the 214 autopsy cases, we excluded 3 subjects whose brain specimens were inadequate for evaluation, and 76 subjects who did not complete the OGTT in 1988. Finally, 135 subjects who underwent both the OGTT and brain autopsy were included in the present study. None of the 135 subjects showed signs of dementia at the clinical examination in 1988. Careful surveillance of cognitive impairment was carried out through a daily monitoring system established by the study team, local practitioners, and the town government.[9,18]

Standard protocol approvals, registrations, and patient consents. The study was approved by the Ethics Committee of the Faculty of Medicine, Kyushu University, and was performed in accordance with the ethical standards described in the 5th revision of the Declaration of Helsinki, 2000. Written informed consent was obtained from all study subjects.

Risk factors. In the clinical examination performed in 1988, the 75-g OGTT was performed after at least a 12-hour overnight fast and the following 3 diabetes-related factors were determined: fasting plasma glucose (FPG), 2-hour post-load plasma glucose (2-hour PG), and fasting insulin. Glucose was determined by the glucose oxidase method and fasting insulin was determined by a radioimmunoassay. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: FPG (mmol/L) × fasting insulin (μU/mL)/22.48155.[20] Blood pressure was measured 3 times at the right upper arm using a mercury sphygmomanometer after at least 5 minutes of rest in a sitting position; the mean of the 3 measurements was used in the analysis. Total cholesterol levels were determined enzymatically. Height and weight were measured in light clothes without shoes, and body mass index (BMI; weight/height squared, kg/m^2) was calculated. Information on exercise and smoking habits was obtained via a standard questionnaire, and these factors were classified as being habitual or not. Regular exercise means engaging in sports or other forms of exertion regularly more than 3 times per week during leisure time. *APOE* genotyping was determined by direct sequencing at Takara Bio Inc., Japan. No homozygous ϵ4 genotype was found among these participants, and those who carried 1 copy of the ϵ4 allele were categorized as *APOE* ϵ4 carriers.

Assessment of neuropathologic changes. The assessment of AD pathology was conducted according to the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) guidelines and Braak stage established by Braak and Braak.[21-23] Brains were fixed in 10% buffered formalin for at least 2 weeks. Brain specimens in each case included the middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, anterior cingulated gyrus, amygdala, hippocampus with entorhinal and transentorhinal cortex (at the level of the lateral geniculate body), calcarine cortex, basal ganglia including the nucleus basalis of Meynert, thalamus, substantia nigra, locus ceruleus, and dorsal vagal nucleus. Sections were embedded in paraffin and were routinely stained using hematoxylin-eosin, Klüver-Barrera, and a modified Bielschowsky method. Specimens from each subject were immunostained with antibodies against phosphorylated tau (AT8, mouse monoclonal, 1:500; Innogenetics, Belgium). Immunolabeling was detected using a standard indirect immunoperoxidase method and visualized using diaminobenzidine (Dojindo, Japan) as a chromogen. The frequency of NPs defined by the CERAD criteria were semiquantitatively categorized into the following 4 groups: none (score 0), sparse (score 1), moderate (score 2), and frequent (score 3). The extent of NFTs according to Braak stage was semiquantitatively classified into the following 4 groups: stage 0, stage I to II, stage III to IV, and stage V to VI. For the pathologic assessment of cerebrovascular diseases, any types of cerebral infarctions and hemorrhages were registered according to gross examination and microscopic assessment, regardless of clinical features. This factor was classified as being present or not.

Statistical analyses. Statistical analyses were conducted using SAS software version 9 (SAS Institute, Cary, NC). Mean or geometric mean values of the diabetes-related factors among the groups of NPs or NFTs were calculated and compared by analysis of covariance, with adjustment for age at clinical examination and sex. We used logistic regression analysis to determine relationships between the risk factors (diabetes-related factors, *APOE* genotype, and their interaction) and pathologic outcome (presence or absence of NPs and NFTs) and are expressed as odds ratios (OR) and 95% confidence intervals (CI). Continuous variables (FPG, fasting insulin, and HOMA-IR) were divided into 3 groups to compare the risk of NPs among tertiles. Missing values (1 for fasting insulin, 1 for HOMA-IR, 6 for *APOE* ϵ4 carrier, and 1 for the grading of Braak stage) were excluded from the analysis. Age at clinical examination was used for adjustment in the present study; adjustment for age at death resulted in equivalent statistical outcomes. Significance was de-

Table 1 Demographic characteristics of the study subjects (n = 135)[a]

Variables	Values
Male sex	54.8
Age at medical examination, y	67.0 ± 9.5
Fasting plasma glucose, mmol/L	5.9 ± 1.2
2-hour post-load plasma glucose, mmol/L	8.3 ± 4.3
Fasting insulin, μU/mL[b,c]	5.2 (2.0–13.6)
HOMA-IR[b,c]	1.3 (0.5–4.0)
Systolic blood pressure, mm Hg	138.7 ± 23.6
Diastolic blood pressure, mm Hg	76.5 ± 12.1
Serum total cholesterol, mmol/L	5.2 ± 1.1
BMI, kg/m^2	22.0 ± 3.2
Current smoking	32.6
Regular exercise[d]	11.1
APOE ε4 carrier[c]	19.4

Abbreviations: BMI = body mass index; HOMA-IR = homeostasis model assessment of insulin resistance.
[a] Values are means ± SD or percentage.
[b] Geometric means and 95% prediction intervals are shown for fasting insulin and HOMA-IR due to their skewed distributions.
[c] Missing values: 1 for fasting insulin, 1 for HOMA-IR, and 6 for *APOE* ε4 carrier.
[d] Engaging in sports or other forms of exertion regularly more than 3 times per week during leisure time.

fined as $p < 0.05$, and marginal significance was defined as $0.05 \leq p < 0.10$ in statistical analysis.

RESULTS The characteristics of the study subjects at clinical examination in 1988 (n = 135) are described in table 1. Mean ± SD age at clinical examination was 67.0 ± 9.5 and mean ± SD age at death was 79.5 ± 9.3 years, and 54.8% (n = 74) of the subjects were male. Overall, 19.4% (n = 25) of subjects were carrying *APOE* ε4. There was no selection bias regardless of autopsy, according to a comparison of demographic characteristics between our study subjects and those who did not undergo autopsy (data not shown). Out of the 135 subjects, 15.6% (n = 21) developed Alzheimer-type dementia. Based on the assessment of AD pathology, the frequencies of NPs were categorized into the following 4 groups by CERAD criteria: 34.8% (n = 47) for none (score 0), 17.0% (n = 23) for sparse (score 1), 14.1% (n = 19) for moderate (score 2), and 34.1% (n = 46) for frequent (score 3). The frequencies of NFTs were classified into the following 4 groups by Braak stage: 14.2% (n = 19) for stage 0, 18.7% (n = 25) for stage I to II, 44.0% (n = 59) for stage III to IV, and 23.1% (n = 31) for stage V to VI. Prevalence of cerebrovascular disease at autopsy was 59.3% (n = 80), which included any types of infarctions (n = 73) and hemorrhages (n = 10).

As shown in table 2, we compared the age- and sex-adjusted mean (or geometric mean) values of diabetes-related factors among groups according to CERAD score for NPs or Braak stage for NFTs. The subjects with NPs (CERAD score 1 to 3) showed significantly higher levels of 2-hour PG, fasting insulin, and HOMA-IR than those without NPs (CERAD score 0). However, there was no obvious dose-response relationship between these variables and CERAD score. The FPG levels remained broadly constant irrespective of CERAD score. Regarding the frequencies of NFTs, we found no relationship between any diabetes-related factor and Braak stage.

As shown in table 3, we estimated the effect of each diabetes-related factor on the presence of AD pathology using logistic regression analysis. As for NPs, elevated 2-hour PG significantly increased the risk of NPs in the age- and sex-adjusted analysis (model 1). Similarly, hyperinsulinemia and high HOMA-IR were also significant positive risk factors

Table 2 Age- and sex-adjusted means of glucose, insulin, and HOMA-IR according to CERAD score and Braak stage[a]

	Frequency of NPs (CERAD score)				p Value (CERAD score 1–3 vs 0)	Frequency of NFTs (Braak stage)				p Value (Braak stage I–IV vs 0)
	0	1	2	3		0	I, II	III, IV	V, VI	
Fasting plasma glucose, mmol/L	5.7	6.0	6.2	5.9	0.22	5.7	6.1	5.8	6.0	0.38
2-hour post-load plasma glucose, mmol/L	7.2	9.0[c]	9.6[b]	8.7	0.03	7.0	9.2[c]	8.4	8.5	0.13
Fasting insulin, μU/mL	4.6	6.1[b]	5.2	5.6[c]	0.03	5.1	5.0	5.2	5.7	0.81
HOMA-IR	1.2	1.6[b]	1.4	1.4[c]	0.02	1.3	1.4	1.3	1.5	0.62

Abbreviations: CERAD = Consortium to Establish a Registry for Alzheimer's Disease; HOMA-IR = homeostasis model assessment of insulin resistance; NP = neuritic plaque.
[a] Geometric means for fasting insulin and HOMA-IR are shown due to their skewed distributions.
[b] $p < 0.05$, [c] $p < 0.10$ vs CERAD score = 0 or Braak stage = 0.

Table 3 **Odds ratios and 95% confidence intervals for the presence vs absence of neuritic plaques and neurofibrillary tangles[a]**

	OR for presence of NPs (CERAD score 1–3 vs 0)				OR for presence of NFTs (Braak stage I–VI vs 0)			
	Model 1		Model 2		Model 1		Model 2	
	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
Fasting plasma glucose, mmol/L	1.33 (0.86–2.04)	0.20	1.41 (0.88–2.26)	0.15	1.31 (0.72–2.37)	0.38	1.35 (0.74–2.47)	0.33
2-hour post-load plasma glucose, mmol/L	1.66 (1.04–2.63)	0.03	1.71 (1.04–2.80)	0.03	1.58 (0.85–2.93)	0.15	1.67 (0.88–3.17)	0.12
Fasting insulin, μU/mL	1.61 (1.04–2.48)	0.03	2.03 (1.11–3.70)	0.02	1.05 (0.62–1.79)	0.85	1.06 (0.55–2.04)	0.86
HOMA-IR	1.67 (1.08–2.59)	0.02	2.11 (1.18–3.79)	0.01	1.14 (0.66–1.98)	0.64	1.19 (0.62–2.30)	0.60

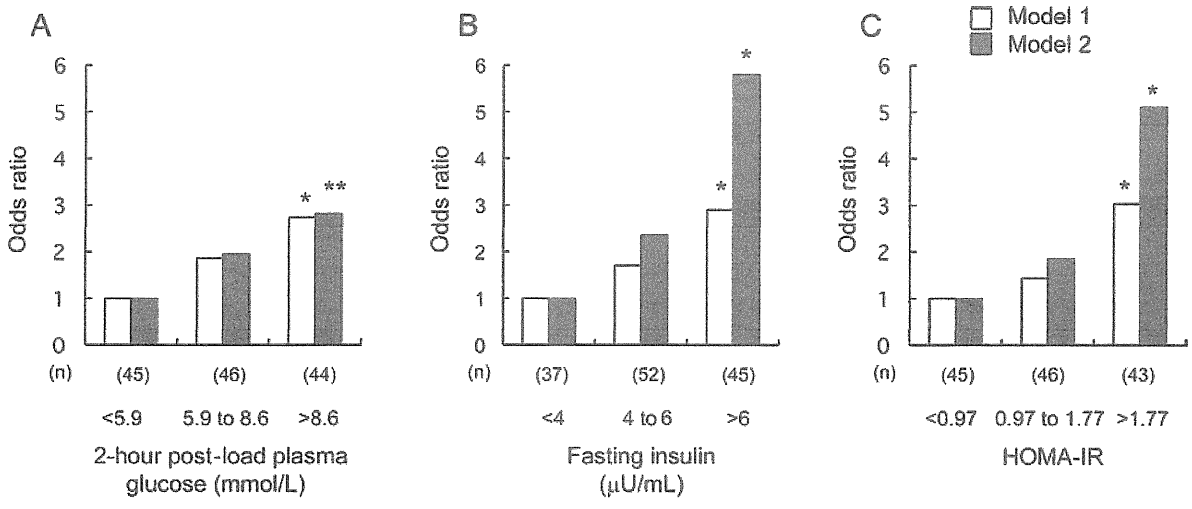
Abbreviations: CERAD = Consortium to Establish a Registry for Alzheimer's Disease; CI = confidence interval; HOMA-IR = homeostasis model assessment of insulin resistance; NP = neuritic plaque; NFT = neurofibrillary tangle; OR = odds ratio.
[a] Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, total cholesterol, body mass index, current smoking, regular exercise, and cerebrovascular disease. ORs are given for each 1-SD increase in glucose, or log fasting insulin and HOMA-IR values.

for NPs. However, there was no relationship between FPG and NPs. These results were almost the same in the multivariate analyses after adjustment for age, sex, systolic blood pressure, total cholesterol, BMI, current smoking, regular exercise, and cerebrovascular disease (model 2). We repeated analyses after excluding the 21 cases with cognitive impairment, and the associations remained unchanged. On the other hand, we found no significant association between diabetes-related factors and NFT pathology (Braak stage I to VI vs stage 0).

To confirm the association between diabetes-related factors and NPs, we compared the risk of NPs among tertiles of 2-hour PG, fasting insulin, and HOMA-IR (figure 1). Compared with the lowest tertile of 2-hour PG (<5.9 mmol/L), the risk of NPs was significantly increased in the highest tertile (>8.6 mmol/L) after adjustment for age and sex (model 1). After adjustment for the aforementioned confounding factors (model 2), this relationship was marginally significant. On the other hand, the highest tertiles of fasting insulin (>6 μU/mL) and HOMA-IR (>1.77) showed increased risk for NPs compared with the lowest tertiles (<4 μU/mL for insulin, <0.97 for HOMA-IR) in models 1 and 2.

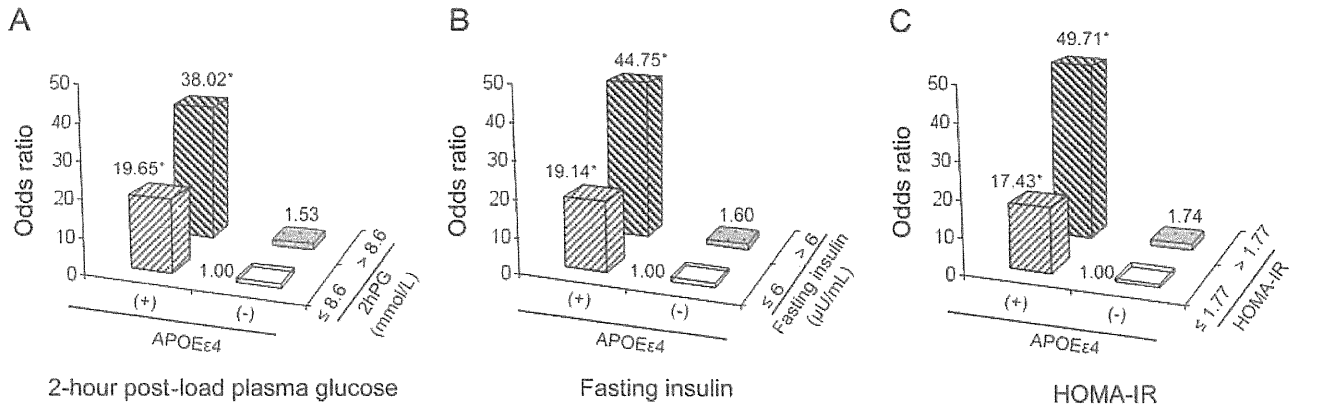
Finally, we examined the combined effects of *APOE* genotype and the magnitude of the diabetes-related factors on the risk of NP pathology (figure 2). For example, the subjects were classified into the following 4 groups according to the 2-hour PG level

Figure 1 **Odds ratios for each tertile of glucose (A), insulin (B), and HOMA-IR (C) vs the lowest tertile for the presence of neuritic plaques**



Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, systolic blood pressure, total cholesterol, body mass index, current smoking, regular exercise, and cerebrovascular disease. *$p < 0.05$, **$p < 0.10$ vs the lowest tertile. HOMA-IR = homeostasis model assessment of insulin resistance.

Figure 2 **Odds ratios for the presence of neuritic plaques according to diabetes-related risk factors and *APOE* genotype**



Adjusted for age, sex, and total cholesterol. The numbers in the figure are odds ratios vs the reference group (*APOE* ϵ*4* noncarrier and lower level of glucose [A], insulin [B], or HOMA-IR [C]). *$p < 0.05$ vs reference group. 2hPG = 2-hour post-load plasma glucose; HOMA-IR = homeostasis model assessment of insulin resistance.

and *APOE* status: low 2-hour PG (lowest and second tertiles, ≤8.6 mmol/L) and noncarriers of *APOE* ϵ*4* (group 1), high 2-hour PG (highest tertile, >8.6 mmol/L) and noncarriers of *APOE* ϵ*4* (group 2), low 2-hour PG and *APOE* ϵ*4* carriers (group 3), and high 2-hPG and *APOE* ϵ*4* carriers (group 4). The ORs for the presence of NPs in these 4 groups were 1.0 in group 1 (reference), 1.5 in group 2, 19.7 in group 3, and 38.0 in group 4. As a result, the coexistence of hyperglycemia and *APOE* ϵ*4* genotype (group 4) was associated with the greatest risk for NPs. We performed similar analyses with fasting insulin and HOMA-IR, and similar patterns were observed.

DISCUSSION We suggest that hyperglycemia, hyperinsulinemia, and insulin resistance are risk factors for NP pathology in AD, and might affect the initiation of NP formation. The lack of a dose-response relationship, and the absence of a significant association between the diabetes-related factors and NFT pathology, might be due to an epidemiologic competing effect, indicating that subjects with very high diabetes-related factors at the clinical examination in 1988 probably died earlier as a result of cardiovascular disease, for example. Nevertheless, NFT pathology was less associated with diabetes-related factors, and NFT pathology is considered to be a consequence of β-amyloid deposition in the amyloid cascade hypothesis.[24] The diabetes-related factors may act upstream of the cascade, and might trigger the AD pathogenesis.

Type 2 diabetes is based on insulin resistance and involves chronic compensatory hyperinsulinemia and hyperglycemia. Insulin itself may affect amyloid metabolism, which leads to NP formation. An impaired insulin signaling may exacerbate β-amyloid accumulation by a weakened inhibition on glycogen synthase kinase 3 (GSK3), which is thought to be critically involved in AD pathogenesis.[25] Activated GSK3 triggers γ-secretase activity[26] and increases β-amyloid production.[27] Alternatively, excessive β-amyloid can be cleared by endocytosis or through direct extracellular proteolytic degradation by insulin-degrading enzyme (IDE).[28] Insulin seems to inhibit the extracellular degradation of β-amyloid by competition for IDE.[29] Furthermore, several lines of evidence suggest that the toxic effects of hyperglycemia can lead to slowly progressive functional and structural abnormalities in the brain.[30] It is possible that vascular factors induced by metabolic disturbance may modify the AD-related pathology, however, the positive association between diabetes-related factors and NP pathology still remained even after the adjustment for cerebrovascular lesions in our study.

On the contrary, insulin is known to facilitate memory in normal physiology, as demonstrated when administered at optimal doses and in the context of sufficient glucose availability.[31] The formation of NPs, as described above, is a hallmark of AD, which refers to the pathologic entity; meanwhile, Alzheimer dementia, which refers to clinical dementia, may also be caused in part by deficiencies in intracellular and intercellular signaling.[32] Insulin resistance affects insulin signaling, which might lead to a decline in cognitive function. In this study, the subjects who developed Alzheimer dementia were far less than those who manifested NPs (n = 21 vs 88); therefore, the present pathology-based study should overlap, but is also distinct from the previously reported clinicoepidemiologic studies.[2,4,5,8,9] Our target in this study was to evaluate how diabetes affects the neuropathologic process of AD, which would precede the cognitive decline.

Four previous studies have examined the association between diabetes and AD-related pathology, but their results are inconsistent.[5,33-35] Of these, the Honolulu-

Asia Aging Study was the only population-based study and reported that participants with type 2 diabetes and the *APOE* ϵ4 allele had a higher number of hippocampal NPs and NFTs in the cortex and hippocampus than those without diabetes and the ϵ4 allele.[5] In our study, the combination of the unfavorable status afforded by the diabetes-related factors and the presence of the ϵ4 allele was associated with NP formation, but not with NFT formation (data not shown). The discrepancy in these studies may reflect differences in design of these studies. One possibility is the difference in the observation period between the evaluation of diabetes and the autopsy. Because the observation period in our study was relatively long (10–15 years) compared with the Honolulu-Asia Aging Study (<8 years), our study design might reduce the possibility of reverse causality that the presence of AD might affect lifestyle of the subjects and the severity of glucose intolerance. Another possibility is the difference in the study subjects. Both studies were population-based and included Asian subjects; however, the mean age at clinical examination of the Honolulu-Asia Aging Study (78 years) was greater than that in our study. The other 3 studies[33-35] reported controversial or statistically insignificant results between diabetes status and AD pathology, probably due to the facility-based design and different races.

Our study suggests that the combination of each diabetes-related factor and the *APOE* ϵ4 genotype may have a synergistic effect on the risk of NPs, even though we failed to show a statistically positive interaction (p for interaction = 0.90 [2-hour PG], 0.84 [fasting insulin], 0.79 [HOMA-IR]). The Honolulu-Asia Aging Study[5] also showed synergistic effects of diabetes and the *APOE* ϵ4 genotype on AD pathology; however, that study did not account for some diabetes-related factors such as insulin levels and HOMA-IR. It was found that apolipoprotein E2 and E3, but not E4, may be involved in β-amyloid clearance.[36] Additionally, apolipoprotein E is commonly colocalized with β-amyloid in NPs,[37] which led to the hypothesis that apolipoprotein E may be involved in β-amyloid aggregation and plaque formation. Because the apolipoprotein E4 isoform stimulates the nucleation and aggregation of β-amyloid in an isoform-specific manner and does not significantly affect the accumulation of β-amyloid deposits,[38] both apolipoprotein E4 and diabetes-related factors may act synergistically on the initiation of β-amyloid aggregation. We consider that a future study using a larger sample size is needed to investigate the interaction between each diabetes-related factor and the *APOE* genotype on the risk of AD pathology.

There are some limitations to our present study. First, the crude, semiquantitative evaluation of NPs (CERAD) and NFTs (Braak stage) could affect the statistical analyses. Second, the medical history of diabetes, such as disease duration, glucose control, and complications, were not considered in this study. Despite these limitations, our study has several strengths. We included community-based subjects, who had detailed metabolic characterization at midlife based on comprehensive blood testing, which included 75-g OGTT profiles and fasting insulin levels, and we systematically assessed AD pathology. Accordingly, the data included in this study are of value to examine the metabolic risk factors for AD pathology. In the Hisayama Study, both participation rate of clinical examinations and autopsy rate have remained at high levels. Therefore, our results could apply to other Japanese populations.

Further studies are required to determine if there is a causal link between insulin resistance and the development of NPs or other AD-related neuropathologies. In the future, adequate control of diabetes might contribute to a strategy for the prevention of AD.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. T. Matsuzaki.

ACKNOWLEDGMENT

The authors thank S. Nagae and K. Sato for technical assistance.

DISCLOSURE

Dr. Matsuzaki reports no disclosures. Dr. Sasaki receives research support from the Ministry of Health, Labor and Welfare of Japan. Dr. Tanizaki, Dr. Hata, Dr. Fujimi, Dr. Matsui, Dr. Sekita, and Dr. Suzuki report no disclosures. Dr. Kanba has served on scientific advisory boards for Eli Lilly and Company, GlaxoSmithKline, Pfizer Inc., Mitsubishi Tanabe Pharma Corporation, Ono Pharmaceutical Co. Ltd., Astellas Pharma Inc., Asahi Kasei Corporation, Shionogi & Co., Ltd., and Otsuka Pharmaceutical Co., Ltd.; has served on the editorial boards of *Molecular Psychiatry*, the *Journal of Neuroscience and Psychiatry*, the *Asian Journal of Psychiatry*, and the *Asia Pacific Journal of Psychiatry*; has received speaker honoraria from Eli Lilly and Company, GlaxoSmithKline, Pfizer Inc., Asahi Kasei Corporation, Janssen, Tsumura & Co., Ajinomoto Co., Inc., Mitsubishi Tanabe Pharma Corporation, Meiji Techno Co. Ltd., Kyowa Hakko Kirin Pharma, Inc., Dainippon Sumitomo Pharma Co., Ltd., Organon International (Schering-Plough Corp.), Otsuka Pharmaceutical Co., Ltd., and Astellas Pharma Inc.; and has received research support from Eli Lilly & Co., GlaxoSmithKline, Pfizer Inc., Asahi Kasei Corporation, Janssen, Tsumura & Co., Ajinomoto Co., Inc., Mitsubishi Tanabe Pharma Corporation, Meiji Techno Co. Ltd., Kyowa Hakko Kirin Pharma, Inc., Dainippon Sumitomo Pharma Co., Ltd., Organon International (Schering-Plough Corp.), Otsuka Pharmaceutical Co., Ltd., and the Ministry of Health, Labor and Welfare of Japan and the Ministry of Education, Culture, Sports, Science and Technology of Japan. Dr. Kiyohara receives research support from the Ministry of Health, Labor and Welfare of Japan. Dr. Iwaki receives research support from the Ministry of Education, Culture, Sports, Science and Technology of Japan and serves on the editorial boards of *Neuropathology*, *Brain Tumor Pathology*, *Pathology—Research and Practice*, and *Pathology International*.

Received October 5, 2009. Accepted in final form February 19, 2010.

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J Epidemiol 2011;21(2):114-121
doi:10.2188/jea.JE20100079

Original Article

Use of Doubly Labeled Water to Validate a Physical Activity Questionnaire Developed for the Japanese Population

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Received May 21, 2010; accepted November 10, 2010; released online January 18, 2011

ABSTRACT

Background: No study has attempted to use the doubly labeled water (DLW) method to validate a physical activity questionnaire administered to a Japanese population. The development and refinement of such questionnaires require that physical activity components related to physical activity level be examined.

Methods: Among 226 Japanese men and women 20 to 83 years of age, total energy expenditure (TEE) was assessed using the Japan Arteriosclerosis Longitudinal Study Physical Activity Questionnaire (JALSPAQ), and the results were compared with TEE measured by the DLW method as a gold standard. Resting metabolic rate (RMR) was measured using the Douglas Bag method.

Results: The median TEE by DLW and physical activity level (PAL: TEE/RMR) were 11.21 MJ/day and 1.88, respectively, for men, and 8.42 MJ/day and 1.83 for women. JALSPAQ slightly underestimated TEE: the differences in mean and standard error were -1.15 ± 1.92 MJ/day. JALSPAQ and DLW TEE values were moderately correlated (Spearman correlation = 0.742, $P < 0.001$; intraclass correlation coefficient = 0.648, $P < 0.001$), and the 95% limit of agreement was −4.99 to 2.69 MJ. Underestimation of TEE by JALSPAQ was greater in active subjects than in less active subjects. Moderate and vigorous physical activity and physical activity during work (ie, occupational tasks and housework) were strongly related to physical activity level. However, the physical activity components that differentiated sedentary from moderately active subjects were not clear.

Conclusions: Physical activity level values on JALSPAQ and DLW were weakly correlated. In addition, estimation of TEE in active subjects should be improved, and the use of a questionnaire to differentiate activity in sedentary and moderately active subjects must be reassessed.

Key words: physical activity questionnaire; doubly labeled water; physical activity; energy expenditure

INTRODUCTION

Accurate assessment of physical activity level is fundamental in epidemiological studies that examine the effect of physical activity on disease prevention and health promotion. Although there are several methods for estimating physical activity level, questionnaires are the most common assessment tool in such studies. Many types of physical activity questionnaires are used in epidemiological studies, but a validation study of such questionnaires suggested that the reliability and validity of measurements of habitual physical activity are quite low.[1–3] In addition, Neilson et al suggested that the ability of physical activity questionnaires to predict total energy expenditure (TEE) is limited. Westerterp et al suggested that questionnaires are satisfactory as an instrument for ranking physical activity level, but not as tools for assessing absolute TEE.[4] We previously examined the International Physical Activity Questionnaire (IPAQ) and reported that it was difficult to distinguish sedentary from moderately active individuals in the Japanese population.[5] Although the IPAQ was developed for international use, we maintain that questionnaires designed to suit each country or culture would increase the validity of assessments of physical activity level. The Japan Arteriosclerosis Longitudinal Study Physical Activity Questionnaire (JALSPAQ) was developed to assess physical activity in the Japan Arteriosclerosis Longitudinal Study.[6,7] This questionnaire was developed using data from physical activity records for the Japanese

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population and included detailed questions on occupational work, housework, and leisure-time physical activity.

The doubly labeled water (DLW) method is an excellent method for measuring TEE in free-living subjects over a period of 1 to 2 weeks[8] and is often used as a gold standard to validate field methods of assessing physical activity levels. However, to our knowledge, only our previous study[5] has used it to examine the validity of a questionnaire used for the Japanese population.

The primary objective of this study was to use the DLW method as the gold standard to validate a physical activity questionnaire developed for the Japanese population. To aid in the development of a valid physical activity questionnaire for Japanese, the secondary objective was to identify the physical activity component that had the greatest impact on physical activity level (PAL).

METHODS

Subjects

The study participants were 226 Japanese men and women age 20 to 83 years (mean ± standard deviation, 50.4 ± 17.1 years) who volunteered at community health care centers and workplaces or enrolled via the internet homepage of our institute. The inclusion criteria of the present study were as follows: absence of any condition affecting energy or water metabolism (eg, thyroid or kidney disease), not pregnant or breast-feeding, residence in home prefecture 2 weeks before and during the study, not on weight-loss or treatment diet, and not consuming more than 40 grams of alcohol per day. The occupations of the participants were homemaker ($n = 59$), office worker ($n = 57$), shipbuilder ($n = 17$), shop assistant ($n = 14$), no regular work ($n = 14$), nurse ($n = 13$), teacher ($n = 11$), salesperson ($n = 11$), factory worker ($n = 6$), clinical examination technician ($n = 5$), physiotherapist ($n = 4$), and other ($n = 12$, cleaner, gardener, dietitian, priest, sports instructor, carpenter, etc.). We were unable to randomly select subjects according to physical activity level. Over the entire assessment period, the participants were carefully instructed to maintain their normal daily activities and eating patterns and to make no conscious effort to lose or gain weight.

Study protocol

This study was approved by the Ethics Committee of the National Institute of Health and Nutrition in Japan. All subjects gave their informed consent in writing before the investigation was begun. TEE was estimated over 1 or 2 weeks, depending on the 2 half-lives of the isotopes used in the DLW method. Body mass and height were measured in the fasting state before administering the dose of DLW and on the last day of the study. On the first day of the study period, baseline urine was collected, and measurements of resting metabolic rate (RMR) and DLW dosing were obtained. The physical activity questionnaire and dietary assessment were completed between the 10th and 12th day of the study period and were checked by the researchers on the last day.

Measurement of resting metabolic rate

Subjects were instructed to refrain from moderate to vigorous physical activity for 24 hours, to fast at least 12 hours, and to get sufficient sleep before the measurements. They were instructed to arrive at the laboratory between 8AM and 9AM. After arrival, they rested quietly in the supine position for 30 minutes before the measurements. Using a mask connected to a Douglas bag, expired gas was collected twice for 10 minutes, with a 1-minute interval between collections. During all RMR measurements, the room temperature was maintained at approximately 24°C. Subjects were lying down and fully awake during the measurements. They were also free from emotional stress and were familiar with the apparatus used. The volume of expired air was measured with a certified gas meter (DC-5, Shinagawa, Tokyo, Japan), the accuracy and precision of which were maintained within 1% of the coefficient of variation (CV). Concentrations of oxygen and carbon dioxide were measured with a mass spectrometer (ARCO-1000, Arco Systems, Chiba, Japan). The precision of expired gas measurement was 0.02% for oxygen and 0.06% for carbon dioxide. RMR was calculated using Weir's equation.[9]

DLW energy measurement

After providing a baseline urine sample, a single dose of approximately 0.06 g/kg body weight of ^2H_2O (99.8 atom%, Cambridge Isotope Laboratories, MA, USA) and 1.4 g/kg body weight of $H_2{}^{18}O$ (10.0 atom%, Taiyo Nippon Sanso, Tokyo, Japan) was given orally to each subject. Then subjects were asked to collect urine samples at 8 predetermined times during the study period, at the same time of day. Except for the baseline collection, all urine samples were collected by the participant, and the time of sampling was recorded. All samples were stored by freezing at −30°C in airtight parafilm-wrapped containers and then analyzed in our laboratory.

Gas samples for the isotope ratio mass spectrometer (IRMS) were prepared by equilibration of the urine sample with a gas. CO_2 was used to equilibrate ^{18}O, and H_2 was used for ^2H. Pt catalyst was used for equilibration of ^2H. The gas sample of the CO_2 and H_2 was analyzed by IRMS (DELTA Plus; Thermo Electron Corporation, Bremen, Germany). Each sample and the corresponding reference were analyzed in duplicate. The average standard deviations for the analyses were 0.5‰ for ^2H and 0.03‰ for ^{18}O. TEE was expressed as mean TEE per day over the study period.

Calculations of isotopic abundance and TEE

The ^2H and ^{18}O zero-time intercepts and elimination rates (k_H and k_O) were calculated using a least-squares linear regression on the natural logarithm of isotope concentration as a function

of the elapsed time from dose administration. Zero-time intercepts were used to determine the isotope pool sizes. Total body water (TBW) was calculated from the mean value of the isotope pool size of ^{2}H divided by 1.041 and that of ^{18}O divided by 1.007. The mean ko/kd of the present study was 1.28 ± 0.06 (range, 1.15–1.56). All ko/kd values were maintained within the recommended range (1.1 to 1.7) for quality control of the analysis, as recommended by the International Atomic Energy Agency.[10] rCO_2 was calculated as follows: $rCO_2 = 0.4554 \times TBW \times (1.007ko - 1.041k_H)$. Calculation of TEE (kcal/day) was performed using a modified Weir's formula based on the CO_2 production rate (rCO_2) and food quotient (FQ).[9] FQ was calculated from the dietary survey during the study period. The calculation assumed that under conditions of perfect nutrient balance, the FQ must equal the respiratory quotient (RQ).[11–13] The average FQ of each occupational group was used for each group (FQ = 0.85–0.95). However, FQ values stratified by occupational group, sex, and age were not significantly different. Physical activity level (PAL) was calculated as TEE/RMR.

Physical activity questionnaire

The physical activity questionnaire developed for the Japan Arteriosclerosis Longitudinal Study (JALSPAQ) was used in this study.[6,7] This questionnaire comprises 14 questions on occupation, locomotion, housework, sleep time, and leisure-time physical activities. In this questionnaire, occupational work was assessed as duration of sitting, standing, walking, and heavy work. Heavy work was defined as lifting more than 10 kg or manual labor of similar intensity. Leisure-time physical activity was assessed by type, duration, and frequency. Questionnaire data were converted to the intensity of each physical activity expressed in metabolic equivalents (METs), according to the Compendium by Ainsworth et al, and summarized as METs·h/day and energy expenditure.[14] In the present study, we used TEE per day, METs·h/day, and PAL as indices of physical activity level from JALSPAQ. Duration of light (<3 METs), moderate (3–5.9 METs), and vigorous (≥6 METs) physical activities was calculated for all physical activities (including occupational activity, housework, and leisure-time physical activity), as well as for leisure-time physical activity only. Working time, including occupational and housework time, was divided into the duration of sitting (<2 METs), standing (2 to <3 METs), walking (3 to <6 METs), and heavy work (≥6 METs), including housework. We calculated the durations of occupational activity and housework together because their frequencies and durations were quite complicated.

Dietary assessment

Dietary habits were assessed by using a brief self-administered diet history questionnaire (BDHQ)—a 4-page structured questionnaire that requested information on the consumption frequencies for a total of 56 food and beverage items, with specified serving sizes described in terms of the servings commonly consumed in the general Japanese population.[15] Energy and macronutrient intakes were calculated using a computer algorithm for the BDHQ, which was based on the Standard Tables of Food Composition in Japan. FQ was calculated by using the equation of Black et al.[11]

Statistical analysis

Statistical analyses were performed using SPSS for Windows (version 16.0J; SPSS Inc., IL, USA). Physical characteristics are classified using the sex and age groups outlined in the Dietary Reference Intake (DRI) of Japan. The estimated energy expenditure data were generally not normally distributed; therefore, medians and interquartile ranges are used to describe these results. Sex and age-group differences were compared using 2-way analysis of covariance. The Bonferroni procedure was used as the post-hoc test. The relation between TEE as estimated by DLW and JALSPAQ was expressed as Spearman correlations, intraclass correlation coefficient (ICC), and 95% limits of agreement (95% LOA: mean difference ± 2 × SD of the mean difference). Bland-Altman plots were also created to evaluate the differences between the 2 methods. To examine the type of physical activities that affected physical activity level, we used 1-way analysis of covariance, Pearson's correlation coefficients, and partial correlation coefficients adjusted for sex and age group.

RESULTS

The physical characteristics of the subjects are shown in Table 1. Body weight did not change significantly during the study period ($P = 0.313$). Among all subjects, 2.8% of men and 6.8% of women were classified as lean (body mass index [BMI] <18.5 kg/m^2), and 31.5% of men and 17.8% of women were classified as obese (BMI >25 kg/m^2) according to the criteria for Japanese.[16] The average TBW was 37.3 ± 7.1 kg in men and 25.9 ± 2.8 kg in women. When 73.2% was defined as the proportion of water in fat-free mass, the percent of fat mass was 24.3 ± 6.1% in men and 33.4 ± 7.0% in women.[17] Three men aged 30 to 49 years had a body weight higher than 100 kg; however, they were fit and their percent of fat mass was less than 25%. In addition, in the assessment of TEE by DLW and JALSPAQ, they did not significantly differ from other subjects.

The medians plus interquartiles for RMR, TEE, and PAL by DLW, TEE by questionnaire, and the differences between the 2 methods are shown by sex and age group in Table 2. The respective medians of TEE and PAL were 11.21 MJ/day and 1.88 for men and 8.42 MJ/day and 1.83 for women. PAL significantly differed by age group, but not by sex. PAL in subjects older than 70 years was significantly higher than in those aged 30 to 49 years ($P = 0.016$) and 50 to 69 years

Table 1. Characteristics of study subjects

Age group, years	*n*	Age (years)	Height (cm)	Body weight			BMI (kg/m^2)	TBW (kg)
				pre (kg)	post (kg)	change (kg)		
Male								
20–29	18	25.0 ± 2.5	171.5 ± 6.0	62.1 ± 7.9	62.3 ± 8.0	0.2 ± 0.7	21.1 ± 2.0	36.4 ± 3.7
30–49	42	36.7 ± 5.3	173.8 ± 6.6	74.8 ± 16.7	74.9 ± 16.6	0.0 ± 1.1	24.6 ± 4.7	41.8 ± 8.3
50–69	31	60.2 ± 6.5	163.8 ± 6.6	63.9 ± 8.1	64.0 ± 8.3	0.1 ± 0.9	23.8 ± 2.4	34.5 ± 4.1
≥70	17	75.1 ± 4.0	162.1 ± 5.0	60.7 ± 8.1	60.8 ± 8.2	0.2 ± 0.9	23.1 ± 2.7	32.0 ± 4.2
Female								
20–29	8	25.3 ± 2.4	157.0 ± 3.9	51.3 ± 2.5	51.2 ± 2.5	−0.1 ± 0.8	20.9 ± 1.6	25.5 ± 1.5
30–49	42	38.7 ± 4.4	158.0 ± 5.4	53.7 ± 8.3	53.7 ± 8.3	0.0 ± 0.7	21.5 ± 3.2	26.9 ± 3.1
50–69	49	62.0 ± 5.1	154.0 ± 4.6	54.6 ± 7.8	54.7 ± 7.9	0.1 ± 0.7	23.0 ± 3.2	25.8 ± 2.7
≥70	19	73.4 ± 3.9	148.0 ± 4.4	50.2 ± 6.1	50.1 ± 6.1	0.1 ± 0.6	22.9 ± 2.8	24.1 ± 2.0

All values are mean ± SD, unless otherwise indicated.
BMI: body mass index; TBW: total body water measured by doubly labeled water method.

Table 2. Resting metabolic rate (RMR) and total energy expenditure (TEE) measured by doubly labeled water (DLW) method and questionnaire

Age group, years		RMR (MJ/day)	TEE by DLW (MJ/day)	PAL	TEE by JALSPAQ (MJ/day)	Difference between DLW and JALSPAQ	
						(MJ/day)	(%)
Male							
	20–29	6.27 (0.92)	12.00 (0.19)	1.89 (0.35)	9.60 (2.12)	−1.69 (2.89)	−15.7 (23.0)
	30–49	6.72 (1.53)	12.88 (4.64)	1.87 (0.45)	11.14 (2.85)	−1.18 (3.30)	−9.5 (20.3)
	50–69	5.50 (1.30)	10.81 (2.11)	2.08 (0.55)	9.18 (1.61)	−2.02 (1.99)	−18.1 (17.5)
	≥70	5.76 (1.41)	11.76 (3.59)	2.11 (0.52)	8.03 (1.65)	−0.97 (2.34)	−12.2 (21.0)
Female							
	20–29	4.73 (0.27)	8.10 (1.18)	1.86 (0.22)	7.43 (1.01)	−1.09 (1.85)	−13.2 (22.3)
	30–49	4.83 (0.82)	8.82 (1.80)	1.84 (0.32)	7.33 (1.75)	−1.26 (1.73)	−14.9 (19.1)
	50–69	4.58 (0.95)	8.53 (1.42)	1.86 (0.37)	8.12 (1.28)	−0.43 (1.76)	−5.3 (20.4)
	≥70	4.62 (0.99)	8.56 (0.86)	1.86 (0.41)	7.08 (1.33)	−0.36 (1.68)	−5.2 (23.3)
P value	Sex	<0.001	<0.001	0.067	<0.001	0.003	0.071
	Age group	<0.001	<0.001	<0.001	<0.001	0.335	0.370
	Sex by age	0.010	0.004	0.481	<0.001	0.591	0.188

All values are median (interquartile), unless otherwise indicated.
PAL: physical activity level (TEE/RMR); JALSPAQ: Japan Arteriosclerosis Longitudinal Study Physical Activity Questionnaire.

($P < 0.001$). JALSPAQ slightly underestimated TEE, with differences in mean and standard error of the mean of −1.15 ± 1.92 MJ/day and −0.020 ± 0.030 MJ/kg/day. TEE values by JALSPAQ and DLW were moderately correlated (Spearman correlation = 0.742, $P < 0.001$; ICC = 0.648, $P < 0.001$). The 95% LOA was −4.99 to 2.69 MJ. The absolute difference between TEE values by DLW and JALSPAQ was significantly greater in men than in women, but the percent difference was not significantly different. The Spearman correlation coefficient and ICC for PAL were 0.423 ($P < 0.001$) and 0.332 ($P < 0.001$), respectively, and the 95% LOA for PAL was −0.86 to 0.46. Use of Bland-Altman plots to compare TEE and PAL by DLW and JALSPAQ suggested that TEE tended to be underestimated in subjects with higher TEE (Spearman correlation, −0.201; $P = 0.002$); however, most values were within the 2 SD of the difference in TEE as determined by the 2 methods (Figure). PAL was not underestimated even in subjects with higher PALs (Spearman correlation, −0.011; $P = 0.866$); however, individual differences were widely distributed.

Using PAL determined using TEE measured by DLW, the subjects were divided into 3 groups according to Dietary Reference Intake (Table 3).[18] The proportions of active (PAL >1.9), moderately active (PAL 1.6 to <1.9), and sedentary (PAL <1.6) individuals were 45.4%, 43.5%, and 11.1% in men, respectively, and 40.7%, 41.5%, and 17.8% in women. TEE by JALSPAQ in the sedentary group was significantly lower than in moderately active and active adults. Total METs assessed by JALSPAQ was lower in sedentary and moderately active individuals than in active individuals. The differences between the 2 methods in the TEE of sedentary and moderately active adults were significantly smaller than in active adults. The total duration of each intensity of physical activity, including occupational and housework activity and leisure-time physical activity, was compared among physical activity levels. The duration of moderate and vigorous

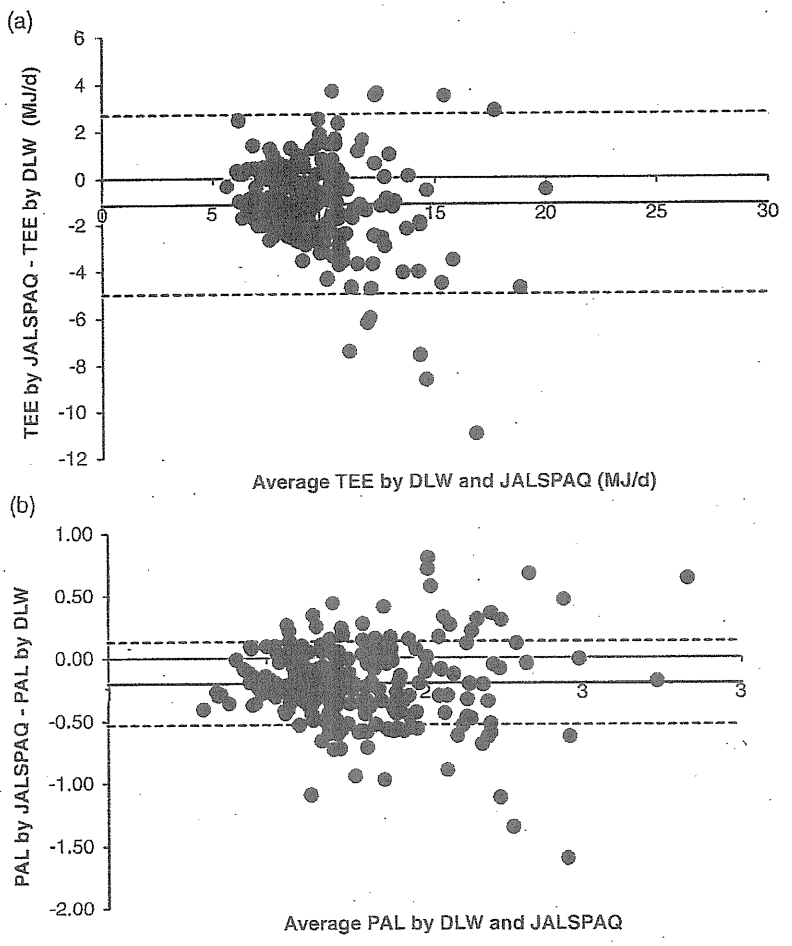

Figure. Bland-Altman plots of total energy expenditure (TEE) and physical activity level (PAL). (a) Comparison of mean TEE estimated by the doubly labeled water (DLW) method and the Japan Arteriosclerosis Longitudinal Study Physical Activity Questionnaire (JALSPAQ), and the difference in TEE as estimated by the 2 methods. (b) Comparison of mean PAL by DLW and JALSPAQ, and the difference in PAL as estimated by the 2 methods. Solid lines indicate the mean difference, and the broken lines indicate 2 SD limits.

physical activity in sedentary and moderately active adults was significantly shorter than in active adults. When we compared only leisure-time physical activity, there was no difference in duration of physical activity. Regarding physical activity during work, duration of walking was significantly shorter in sedentary individuals than in moderately active and active individuals. In addition, walking duration was significantly shorter in moderately active adults than in active adults. The proportion of heavy work differed significantly among groups; greater activity was associated with heavier work.

Regarding the types of physical activity that were correlated with PAL, correlation coefficients and partial correlation coefficients adjusted for sex and age group are shown in Table 4. Duration of total, moderate, and vigorous physical activity were weakly correlated with PAL. However, duration of leisure-time physical activity was not correlated with PAL. During working time, duration of standing, walking, and heavy work were weakly correlated with PAL.

DISCUSSION

This study used the DLW method as a gold standard to examine the validity of a physical activity questionnaire designed for the Japanese population in a large number of subjects with widely varying physical activity levels. With the DLW method as the gold standard, JALSPAQ estimated TEE relatively well, but underestimation was more frequent at higher physical activity levels.

The body height and weight of the present subjects were similar to the standard values for the Japanese population.[18] RMR was also similar to the standard RMR values for the Japanese population presented in Dietary Reference Intake.[18] Thus, we conclude that the present subjects had the general physical characteristics of the Japanese general population. However, the physical activity level of the present subjects was higher than that noted in our previous studies: 42.9% of the present subjects were classified as active, using the definition in the Dietary Reference Intake.[18] We recruited