

**eTable 2.** Associations between HOMA-IR values and markers of glycemic control divided by median BMI in women ( $n = 760$ ).

**eTable 3.** Associations between HOMA-IR values and higher percentile of HbA1c or FPG or lower percentile of 1,5-AG.

**eTable 4.** Associations between HOMA-IR values and higher percentile of HbA1c or FPG or lower percentile of 1,5-AG.

**eTable 5.** Associations between BMI and markers of glycemic control in multivariate regression model that excluded HOMA-IR.

**eTable 6.** Associations between waist circumference and markers of glycemic control in multivariate regression model that excluded HOMA-IR.

Abstract in Japanese.

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ORIGINAL INVESTIGATION

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# Association of hemoglobin A<sub>1c</sub> and glycated albumin with carotid atherosclerosis in community-dwelling Japanese subjects: the Hisayama Study

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## Abstract

**Background:** It is not clear which glucose measure is more useful in the assessment of atherosclerosis. We investigated the associations of hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>), glycated albumin (GA), 1,5-anhydroglucitol (1,5-AG), fasting plasma glucose (FPG), and 2-hour postload glucose (PG) with carotid intima-media thickness (IMT) in community-dwelling Japanese subjects.

**Methods:** A total of 2702 subjects aged 40–79 years underwent a 75-g oral glucose tolerance test and measurements of HbA<sub>1c</sub>, GA, 1,5-AG, and carotid IMT by ultrasonography in 2007–2008. Carotid wall thickening was defined as a maximum IMT of >1.0 mm. The crude and multivariable-adjusted linear and logistic regression models were used to analyze cross-sectional associations between levels of glycemic measures and carotid IMT.

**Results:** The crude average of the maximum IMT increased significantly with rising quartiles of HbA<sub>1c</sub>, GA, FPG, and 2-hour PG levels in subjects with and without glucose intolerance (GI), while no clear association was observed for 1,5-AG. After adjustment for other confounding factors, positive trends for HbA<sub>1c</sub>, GA, and FPG (all *p* for trend < 0.05), but not 2-hour PG (*p* = 0.07) remained robust in subjects with GI, but no such associations were found in those without GI. When estimating multivariable-adjusted  $\beta$  values for the associations of 1 SD change in glycemic measures with the maximum IMT in subjects with GI, the magnitude of the influence of HbA<sub>1c</sub> ( $\beta$  = 0.021), GA ( $\beta$  = 0.024), and FPG ( $\beta$  = 0.024) was larger than that of 2-hour PG ( $\beta$  = 0.014) and 1,5-AG ( $\beta$  = 0.003). The multivariable-adjusted odds ratios for the presence of carotid wall thickening increased significantly with elevating HbA<sub>1c</sub>, GA, and FPG levels only in subjects with GI (all *p* for trend < 0.001). Among subjects with GI, the area under the receiver operating characteristic curve significantly increased by adding HbA<sub>1c</sub> (*p* = 0.04) or GA (*p* = 0.04), but not 1,5-AG, FPG, or 2-hour PG, to the model including other cardiovascular risk factors.

**Conclusions:** In community-dwelling Japanese subjects with GI, elevated HbA<sub>1c</sub>, GA, and FPG levels were significantly associated with increased carotid IMT, and HbA<sub>1c</sub> and GA provided superior discrimination for carotid wall thickening compared to 1,5-AG, FPG, and 2-hour PG, suggesting that HbA<sub>1c</sub> and GA are useful for assessing carotid atherosclerosis.

**Keywords:** Hemoglobin A<sub>1c</sub>, Glycated albumin, 1,5-anhydroglucitol, Fasting plasma glucose, 2-hour postload glucose, Carotid atherosclerosis

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## Background

It has been well established that hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) levels, which have been used widely as a measure of chronic hyperglycemia, are closely associated with the risk of microvascular complications, such as diabetic retinopathy, and this fact has led to the adoption of HbA<sub>1c</sub> as a diagnostic tool for diabetes by the International Expert Committee [1]. The question has now arisen as to whether HbA<sub>1c</sub> measurement is useful in the assessment of macrovascular complications. Several population-based epidemiological studies in Asian populations [2–5] as well as in Western populations [6–13] have focused on the association between HbA<sub>1c</sub> levels and intima-media thickness (IMT) of carotid arteries, which is generally accepted as a marker of the early stage of atherosclerosis. However, this issue has not been assessed sufficiently in a general Japanese population [5].

Glycated albumin (GA) and 1,5-anhydroglucitol (1,5-AG) levels, which are serum markers of short-term glycemic status, have also been shown to be intimately related to microvascular complications in recent epidemiological studies, including our previous report [14–16], and there has been a growing interest in GA and 1,5-AG as alternative markers of hyperglycemia [17]. Although several studies have examined the associations of GA [15, 18–25] and 1,5-AG [26, 27] levels with atherosclerosis among patients on dialysis and those with diabetes or hypertension, very few studies have been conducted to clarify this association in general populations [5, 28]. In addition, to our knowledge, there are no data comparing HbA<sub>1c</sub>, GA, 1,5-AG, fasting plasma glucose (FPG), and 2-hour postload glucose (PG) measurements as a tool for the assessment of atherosclerosis.

To investigate which glucose measure is more useful in the assessment of atherosclerosis, we examined the associations of HbA<sub>1c</sub>, GA, 1,5-AG, FPG, and 2-hour PG levels with carotid IMT in a community-dwelling Japanese population stratified by glucose tolerance status, and compared the magnitude of the influence of these five glycemic measures on the ability to discriminate carotid wall thickening.

## Methods

### Study population

A population-based prospective study of cardiovascular disease (CVD) and its risk factors has been underway since 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area on Japan's Kyushu Island. The population of the town has been stable for 50 years and was approximately 8400 in 2010. The age and occupational distributions, and nutritional intake of the population were similar to those of Japan as a whole based on data from the national census and nutrition

survey [29]. In 2007 and 2008, a cross-sectional survey for the present study was performed in the town. A detailed description of this survey was published previously [16, 30]. Among a total of 3835 residents aged 40–79 years based on the town registry, 2957 (77.1 %) took part in a comprehensive assessment, including a 75-g oral glucose tolerance test (OGTT), the measurement of HbA<sub>1c</sub>, and a carotid ultrasound examination. We excluded eight subjects who did not consent to participate in the study, 46 who had already had breakfast, 35 who did not undergo the OGTT due to receiving insulin therapy, and 156 who refused the OGTT, leaving a total of 2712 subjects who completed the OGTT, HbA<sub>1c</sub> measurement, and carotid ultrasound. Among these, 10 subjects without measurement of GA or 1,5-AG were excluded, and the remaining 2702 subjects (1198 men and 1504 women) were enrolled in the present study.

### Clinical evaluation and laboratory measurements

The study subjects underwent the OGTT between 8:00 and 10:30 A.M. after an overnight fast of at least 12 h. Blood for the glucose assay was obtained by venipuncture into tubes containing sodium fluoride at fasting and at 2-hour postload, and was separated into plasma and blood cells within 20 min. Plasma glucose concentrations were determined by the hexokinase method. Glucose tolerance status was classified as normal glucose tolerance (NGT) and glucose intolerance (GI) according to the 1998 World Health Organization criteria [31]; namely, for NGT, FPG <6.1 mmol/L and 2-hour PG <7.8 mmol/L; for GI, FPG ≥6.1 mmol/L, 2-hour PG ≥7.8 mmol/L, or the use of antidiabetic medications. Diabetes was defined as FPG ≥7.0 mmol/L, 2-hour PG ≥11.1 mmol/L, or the use of antidiabetic medications. HbA<sub>1c</sub> levels were measured by latex aggregation immunoassay (Determiner HbA1C; Kyowa Medex, Tokyo, Japan). The values for HbA<sub>1c</sub> were estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value calculated with the formula: HbA<sub>1c</sub> (%) = 1.02 × HbA<sub>1c</sub> (Japan Diabetes Society) (%) + 0.25 % [32]. A portion of each serum specimen was stored at -80 °C for 5 years until it was used for measurement of GA and 1,5-AG in 2012. Serum GA levels were determined by an enzymatic method using an albumin-specific proteinase, ketoamine oxidase, and an albumin assay reagent (Lucica GA-L; Asahi Kasei Pharma, Tokyo, Japan). Serum 1,5-AG concentrations were measured enzymatically (Lana 1,5AG Auto Liquid; Nippon Kayaku, Tokyo, Japan). The intra- and interassay coefficients of variation were 0.6–1.1 % and 1.4–3.4 % for HbA<sub>1c</sub>, 0.5–3.2 % and 1.3 % for GA, 1.1–2.0 % and 2.5–2.8 % for 1,5-AG, and 0.4–1.3 % and 1.9–3.0 % for plasma glucose, respectively. Serum insulin values were measured by a chemiluminescent enzyme immunoassay. The homeostasis model

assessment of insulin resistance (HOMA-IR) was calculated with the formula: FPG (mmol/L)  $\times$  fasting serum insulin ( $\mu$ U/mL) / 22.5 [33], and subjects in the highest quartile of HOMA-IR distribution in our study population (HOMA-IR  $\geq$ 2.07) were defined as having insulin resistance [31]. Serum total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol and triglycerides were all determined enzymatically. Hyper-LDL cholesterolemia was defined as LDL cholesterol levels  $\geq$ 3.62 mmol/L or the use of lipid-lowering medications [34]. Freshly voided urine samples were tested by the dipstick method. Proteinuria was defined as 1+ or more. Serum creatinine concentrations were measured enzymatically, and estimated glomerular filtration rate (eGFR) (mL/min/1.73 m<sup>2</sup>) was calculated using the following modified equations of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) for Japanese [35]: for men with a serum creatinine level  $>$ 0.9 mg/dL,  $eGFR = 141 \times (\text{serum creatinine}/0.9)^{-1.209} \times 0.993^{\text{Age}} \times 0.813$ ; for men with a serum creatinine level  $\leq$ 0.9 mg/dL,  $eGFR = 141 \times (\text{serum creatinine}/0.9)^{-0.411} \times 0.993^{\text{Age}} \times 0.813$ ; for women with a serum creatinine level  $>$ 0.7 mg/dL,  $eGFR = 144 \times (\text{serum creatinine}/0.7)^{-1.209} \times 0.993^{\text{Age}} \times 0.813$ ; for women with a serum creatinine level  $\leq$ 0.7 mg/dL,  $eGFR = 144 \times (\text{serum creatinine}/0.7)^{-0.329} \times 0.993^{\text{Age}} \times 0.813$ . Chronic kidney disease (CKD) was defined as either an eGFR  $<$ 60 mL/min/1.73 m<sup>2</sup> or the presence of proteinuria [36].

The height and weight were measured with the subject in light clothes without shoes, and the body mass index (BMI) (kg/m<sup>2</sup>) was calculated. Blood pressure was obtained three times using an automated sphygmomanometer (BP-203 RVIII; Omron Healthcare Co., Ltd., Kyoto, Japan) with the subject in a sitting position after rest for at least 5 min; the average values were used in the analyses. Hypertension was defined as a systolic blood pressure  $\geq$ 140 mmHg, a diastolic blood pressure  $\geq$ 90 mmHg, or current treatment with antihypertensive agents.

Each participant completed a self-administered questionnaire covering medical history, antidiabetic, antihypertensive, and lipid-lowering treatments, alcohol intake, smoking habits, and physical activity. Alcohol intake and smoking habits were classified as either current use or not. Current smoking was defined as when the subject smoked at least 1 cigarette per day. Current drinking was defined as when the subject consumed at least 1 alcohol beverage per month. Subjects engaging in sports at least three times per week during their leisure time were defined as the regular exercise group. History of CVD was defined as any preexisting events of stroke or coronary heart disease, including myocardial infarction and coronary intervention. All cardiovascular events were adjudicated on the basis of physical examinations

and a review of all available clinical information including medical records and imaging.

#### Carotid ultrasonography

Carotid ultrasound was performed in a supine position using a real-time, B-mode ultrasound imaging unit (Toshiba Sonolayer SSA-250A; Toshiba, Tokyo, Japan) with a 7.5-MHz annular array probe. The ultrasound examination was carried out by specially trained laboratory technicians using a standardized technique. The technicians were blinded to the medical history, and clinical and laboratory data of each participant. The IMT was measured using the long-axis view of each common carotid artery. An image was obtained in the region 20 mm proximal to the origin of the bulb at the far wall of each common carotid artery. The IMT values at 250 computer-based points in the region were measured on each side using a computer-assisted measurement system (Intimascope; Media Cross Co., Ltd., Tokyo, Japan) [37]. The maximum IMT was defined as the largest IMT value in either the left or right common carotid artery, and carotid wall thickening was defined as a maximum IMT of  $>$ 1.0 mm according to the Japan Academy of Neurosonology and the Japan Society of Ultrasonics in Medicine's guidelines [38, 39].

#### Statistical analysis

The SAS software package version 9.3 (SAS Institute, Cary, NC) was used to perform all statistical analyses. The differences in the mean values or frequencies of risk factors between glucose tolerance categories were assessed using the linear or logistic regression model, respectively. HbA<sub>1c</sub>, GA, 1,5-AG, FPG, and 2-hour PG levels were divided into quartiles by the presence or absence of GI. The values of IMT, fasting insulin, HOMA-IR, and triglycerides were transformed into logarithms to improve the skewed distribution, and geometric means were reported by back transformation. The linear regression model was used to examine the associations of 1 SD change in glycemic measures with the maximum IMT. The adjusted average values of the maximum IMT were calculated using the analysis of covariance method, and their trends across the quartiles of glycemic measures were tested by the linear regression model. The crude and multivariable-adjusted odds ratios (ORs) and their 95 % confidence intervals (CIs) for the presence of carotid wall thickening were estimated using the logistic regression model. Because serum 1,5-AG levels are decreased in the presence of hyperglycemia, the highest quartile was used as the reference group for 1,5-AG, while the lowest quartile was defined as the reference category for other glycemic measures. The heterogeneity in the influence of each glycemic measure on carotid wall thickening between subjects with and without other

cardiovascular risk factors was assessed by adding an interaction term to the relevant statistical model. To compare the discrimination for the presence of carotid wall thickening between the models adjusted for known cardiovascular risk factors with and without continuous values of each glycemic measure, the difference in the area under the receiver operator characteristic curve (AUC) among models was estimated using the method of DeLong et al. [40]. A value of  $p < 0.05$  was considered statistically significant in all analyses.

#### Ethical considerations

This study was conducted with the approval of the Kyushu University Institutional Review Board for Clinical Research, and written informed consent was obtained from all the participants.

#### Results

Of the study participants, there were 1603 (59.3 %) with NGT and 1099 (40.7 %) with GI. Among subjects with GI, 145 (13.2 %) had isolated fasting hyperglycemia (FPG  $\geq 6.1$  mmol/L and 2-hour PG  $< 7.8$  mmol/L), 457 (41.6 %) had isolated 2-hour postload hyperglycemia (FPG  $< 6.1$  mmol/L and 2-hour PG  $\geq 7.8$  mmol/L), and 497 (45.2 %) had both fasting and 2-hour postload hyperglycemia (FPG  $\geq 6.1$  mmol/L and 2-hour PG  $\geq 7.8$  mmol/L). The clinical characteristics of subjects are shown by GI status in Table 1. The mean values of age, maximum IMT, HbA<sub>1c</sub>, GA, FPG, 2-hour PG, fasting insulin, HOMA-IR, systolic and diastolic blood pressures, BMI, LDL cholesterol, and triglycerides, and the frequencies of men, insulin resistance, hypertension, antihypertensive and lipid-lowering medications, hyper-LDL cholesterolemia, proteinuria, CKD, and history of CVD were significantly higher in subjects with GI than in those with NGT, and subjects with GI had significantly lower 1,5-AG, HDL cholesterol, and eGFR values. The mean values of total cholesterol and the frequencies of alcohol intake, smoking habits, and regular exercise did not differ between the groups.

We estimated the crude and multivariable-adjusted geometric average of the maximum IMT according to the quartiles of each glycemic measure in subjects with GI. The crude geometric average of the maximum IMT increased significantly with rising HbA<sub>1c</sub>, GA, FPG, and 2-hour PG levels (all  $p$  for trend  $< 0.001$ ), but no clear association was observed for 1,5-AG levels ( $p$  for trend = 0.30). Similar associations were also observed in subjects with NGT ( $p$  for trend  $< 0.05$  for HbA<sub>1c</sub>, GA, FPG, and 2-hour PG;  $p$  for trend = 0.32 for 1,5-AG). Among subjects with GI, positive trends for HbA<sub>1c</sub> ( $p$  for trend = 0.048), GA ( $p$  for trend  $< 0.001$ ), and FPG ( $p$  for trend  $< 0.001$ ), but not 2-hour PG ( $p$  for trend = 0.07), remained significant even after adjustment for cardiovascular risk

factors; namely, age, sex, hypertension, LDL cholesterol, HDL cholesterol, BMI, alcohol intake, smoking habits, regular exercise, and lipid-lowering medication (Fig. 1). Meanwhile, in those with NGT, the significant associations between levels of any glycemic measures and the maximum IMT disappeared after adjustment for the above-mentioned confounding factors (Additional file 1: Figure S1).

In subjects with GI, when continuous variables of glycemic measures were used instead of quartile categories, HbA<sub>1c</sub>, GA, FPG, and 2-hour PG levels were significantly and positively associated with the maximum IMT even after adjustment for other cardiovascular factors, but no such association was seen for 1,5-AG (Table 2). The magnitude of the influence of 1 SD increment in HbA<sub>1c</sub> ( $\beta = 0.021$ ,  $p < 0.001$ ), GA ( $\beta = 0.024$ ,  $p < 0.001$ ), and FPG ( $\beta = 0.024$ ,  $p < 0.001$ ) was larger than that of 2-hour PG ( $\beta = 0.014$ ,  $p = 0.03$ ). In those with NGT, no significant associations were found for all five glycemic measures after adjustment for other confounding factors (Additional file 1: Table S1).

The crude and multivariable-adjusted ORs and their 95 % CIs for the presence of carotid wall thickening according to the quartiles of each glycemic measure in subjects with GI are shown in Table 3. The crude ORs for the presence of carotid wall thickening significantly increased with elevating HbA<sub>1c</sub>, GA, FPG, and 2-hour PG levels, while lower 1,5-AG levels were significantly associated with prevalent carotid wall thickening. These associations remained robust even after adjustment for other confounding factors for HbA<sub>1c</sub>, GA, and FPG (all  $p$  for trend  $< 0.001$ ), but not for 1,5-AG ( $p = 0.09$ ) and 2-hour PG ( $p = 0.14$ ). In subjects with NGT, although the crude OR for carotid wall thickening did not increase with decreasing 1,5-AG levels, HbA<sub>1c</sub>, GA, FPG, and 2-hour PG levels were significantly and positively associated with the presence of carotid wall thickening (Additional file 1: Table S2). However, these upward trends were markedly attenuated and no longer significant after multivariable adjustment.

We next performed stratified analyses to examine whether the associations of glycemic measures with the presence of carotid wall thickening differed between groups with and without other cardiovascular risk factors in subjects with GI (Additional file 1: Figure S2A and S2B). Overall, there were no statistically significant differences in the multivariable-adjusted ORs for the prevalent carotid wall thickening per 1 SD increment in HbA<sub>1c</sub>, GA, FPG, and 2-hour PG, and 1 SD decrement in 1,5-AG according to levels of other risk factors: sex, age ( $< 65$  and  $\geq 65$  years), smoking habits, insulin resistance, hypertension, and hyper-LDL cholesterolemia (all  $p$  for heterogeneity  $> 0.05$ ).

To evaluate the improvement in discriminative ability for detecting the presence of carotid wall thickening by

**Table 1** Clinical characteristics of subjects, 2007–2008

	Total n = 2702	Glucose tolerance status		P value
		Normal n = 1603	Glucose intolerance n = 1099	
Age (years)	60 (10)	58 (11)	63 (9)	<0.001
Men (%)	44.3	37.5	54.3	<0.001
Maximum IMT (mm)	0.97 (0.96 to 0.98)	0.94 (0.93 to 0.95)	1.01 (1.00 to 1.02)	<0.001
Hemoglobin A <sub>1c</sub> (%)	5.5 (0.7)	5.2 (0.4)	5.9 (0.9)	<0.001
(mmol/mol)	37 (8)	33 (4)	41 (10)	<0.001
Glycated albumin (%)	15.2 (2.8)	14.4 (1.3)	16.4 (3.7)	<0.001
1,5-anhydroglucitol (μg/mL)	20.2 (8.3)	22.2 (7.4)	17.3 (8.7)	<0.001
Fasting plasma glucose (mmol/L)	5.8 (1.2)	5.3 (0.4)	6.5 (1.5)	<0.001
2-hour postload glucose (mmol/L)	8.0 (3.7)	6.0 (1.1)	10.8 (4.3)	<0.001
Fasting insulin (pmol/L)	39.3 (38.5 to 40.2)	34.6 (33.7 to 35.5)	47.3 (45.7 to 49.0)	<0.001
HOMA-IR	1.39 (1.36 to 1.42)	1.13 (1.10 to 1.16)	1.87 (1.80 to 1.94)	<0.001
Insulin resistance (%)	24.9	12.9	42.5	<0.001
Diabetes (%)	15.4	0	37.9	0.93
Antidiabetic medication (%)	6.4	0	15.7	0.93
Systolic blood pressure (mmHg)	131 (19)	126 (18)	138 (18)	<0.001
Diastolic blood pressure (mmHg)	80 (11)	77 (11)	83 (10)	<0.001
Hypertension (%)	45.6	33.0	64.0	<0.001
Antihypertensive medication (%)	28.5	19.0	42.4	<0.001
BMI (kg/m <sup>2</sup> )	23.2 (3.4)	22.5 (3.1)	24.3 (3.5)	<0.001
Total cholesterol (mmol/L)	5.45 (0.93)	5.44 (0.94)	5.47 (0.93)	0.42
LDL cholesterol (mmol/L)	3.24 (0.80)	3.21 (0.80)	3.29 (0.81)	0.02
HDL cholesterol (mmol/L)	1.73 (0.46)	1.81 (0.47)	1.62 (0.43)	<0.001
Triglycerides (mmol/L)	1.21 (1.18 to 1.23)	1.08 (1.06 to 1.11)	1.41 (1.36 to 1.46)	<0.001
Hyper-LDL cholesterolemia (%)	42.5	37.1	50.3	<0.001
Lipid-lowering medication (%)	14.3	9.5	21.3	<0.001
Current drinking (%)	51.9	51.2	52.9	0.38
Current smoking (%)	21.2	21.5	20.8	0.67
Regular exercise (%)	12.4	12.0	12.9	0.46
eGFR (mL/min/1.73 m <sup>2</sup> )	76.3 (10.9)	77.4 (10.4)	74.7 (11.5)	<0.001
Proteinuria (%)	5.0	2.9	8.2	<0.001
Chronic kidney disease (%)	10.8	7.8	15.1	<0.001
History of CVD (%)	4.4	2.8	6.6	<0.001

Maximum IMT, fasting insulin, HOMA-IR, and triglycerides values are shown by geometric means and 95 % confidence intervals due to the skewed distribution. All other values are given as the mean (SDs) or as a percentage.

Insulin resistance was defined as HOMA-IR  $\geq 2.07$  (the highest quartile of HOMA-IR in total study population).

Diabetes was defined as fasting plasma glucose  $\geq 7.0$  mmol/L, 2-hour postload glucose  $\geq 11.1$  mmol/L, or the use of antidiabetic medications.

Hypertension was defined as blood pressure  $\geq 140/90$  mmHg or current use of antihypertensive agents.

Hyper-LDL cholesterolemia was defined as LDL cholesterol  $\geq 3.62$  mmol/L or the use of lipid-lowering medications.

Proteinuria was defined as 1+ or more.

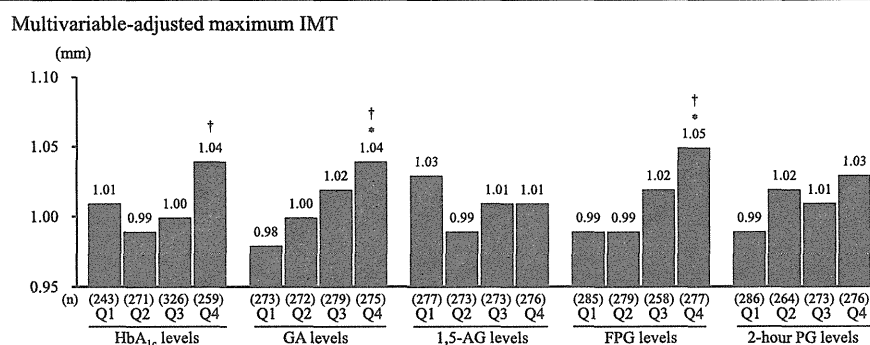
Chronic kidney disease was defined as either an eGFR  $< 60$  mL/min/1.73 m<sup>2</sup> or the presence of proteinuria.

Regular exercise was defined as engaging in sports at least three times per week during leisure time.

IMT intima-media thickness, HOMA-IR homeostasis model assessment of insulin resistance, BMI body mass index, LDL low-density lipoprotein, HDL high-density lipoprotein, eGFR estimated glomerular filtration rate, CVD cardiovascular disease.

addition of continuous values of each glycemic measure to the model including potential cardiovascular risk factors, we compared the AUC between the models with

and without each glycemic measure among subjects with GI (Table 4). The AUC increased significantly by adding HbA<sub>1c</sub> (0.729,  $p = 0.04$ ) or GA (0.728,  $p = 0.04$ ) values to



**Fig. 1** Multivariable-adjusted geometric average of maximum intima-media thickness according to quartiles of each glycemic measure in subjects with glucose intolerance. IMT: intima-media thickness; HbA<sub>1c</sub>: hemoglobin A<sub>1c</sub>; GA: glycated albumin; 1,5-AG: 1,5-anhydroglucitol; FPG: fasting plasma glucose; 2-hour PG: 2-hour postload glucose. \*  $p < 0.01$  vs Quartile 1, †  $p$  for trend  $< 0.05$ . Multivariable adjustment was made for age, sex, hypertension, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body mass index, alcohol intake, smoking habits, regular exercise, and lipid-lowering medication. HbA<sub>1c</sub>: Q1,  $< 5.4$  (36); Q2, 5.4–5.6 (36–38); Q3, 5.7–6.2 (39–44); Q4,  $\geq 6.3$  % (45 mmol/mol); GA: Q1,  $< 14.3$ ; Q2, 14.3–15.3; Q3, 15.4–17.1; Q4,  $\geq 17.2$  %; 1,5-AG: Q1,  $< 11.0$ ; Q2, 11.0–16.9; Q3, 17.0–22.9; Q4,  $\geq 23.0$   $\mu\text{g/mL}$ ; FPG: Q1,  $< 5.7$ ; Q2, 5.7–6.1; Q3, 6.2–6.8; Q4,  $\geq 6.9$  mmol/L; 2-hour PG: Q1,  $< 8.0$ ; Q2, 8.0–9.1; Q3, 9.2–12.3; Q4,  $\geq 12.4$  mmol/L

the model including the potential cardiovascular risk factors mentioned above (0.719). When 1,5-AG (0.721,  $p = 0.27$ ), FPG (0.724,  $p = 0.19$ ), and 2-hour PG (0.721,  $p = 0.39$ ) values were added, the AUC did not increase significantly.

We also performed sensitivity analyses excluding subjects with history of CVD and found that the findings were not substantially altered (data not shown).

## Discussion

Using data from a cross-sectional survey in a Japanese community, we demonstrated that in subjects with GI, elevated levels of HbA<sub>1c</sub>, GA, and FPG were more strongly associated with increased maximum IMT compared to 1,5-AG and 2-hour PG, independent of other cardiovascular risk factors. Similar results were obtained from analysis of the associations between glycemic measures and the presence of carotid wall thickening. There were no clear differences in the influence of each glycemic measure between subjects with and without other

cardiovascular risk factors. Furthermore, adding HbA<sub>1c</sub> or GA to a model including known cardiovascular risk factors significantly improved the assessment of the likelihood of prevalent carotid wall thickening in those with GI. These findings suggest that HbA<sub>1c</sub> and GA provide superior discrimination for the presence of carotid wall thickening, which is an early stage of atherosclerosis, compared to other glycemic measures in community-dwelling Japanese subjects with GI.

In the present study, increased HbA<sub>1c</sub> levels were significantly and positively associated with the presence of carotid wall thickening in individuals with GI, independently of other cardiovascular risk factors. Other Asian population studies have also shown that higher HbA<sub>1c</sub> levels were significantly and independently related to increased carotid IMT in Chinese populations [2, 3] and an Asian Indian population [4]. Similar findings were observed in most studies in Western populations [6, 8–10, 13]. With regard to clinical CVD events, although some cohort studies in the U.S. have demonstrated

**Table 2** The associations of 1 SD increment in HbA<sub>1c</sub>, GA, FPG, and 2-hour PG, and 1 SD decrement in 1,5-AG with the maximum IMT in subjects with glucose intolerance

Glycemic measures	Crude		Multivariable-adjusted	
	$\beta$ (95 % CI)	$P$ value	$\beta$ (95 % CI)	$P$ value
HbA <sub>1c</sub> , per 0.9 % (10 mmol/mol) increment	0.028 (0.015 to 0.041)	$< 0.001$	0.021 (0.009 to 0.034)	$< 0.001$
GA, per 3.7 % increment	0.036 (0.023 to 0.049)	$< 0.001$	0.024 (0.012 to 0.036)	$< 0.001$
1,5-AG, per 8.7 $\mu\text{g/mL}$ decrement	0.004 (-0.010 to 0.017)	0.59	0.003 (-0.009 to 0.015)	0.61
FPG, per 1.5 mmol/L increment	0.030 (0.017 to 0.043)	$< 0.001$	0.024 (0.011 to 0.036)	$< 0.001$
2-hour PG, per 4.3 mmol/L increment	0.029 (0.016 to 0.043)	$< 0.001$	0.014 (0.002 to 0.026)	0.03

Multivariable adjustment was made for age, sex, hypertension, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body mass index, alcohol intake, smoking habits, regular exercise, and lipid-lowering medication

HbA<sub>1c</sub> hemoglobin A<sub>1c</sub>, GA glycated albumin, 1,5-AG 1,5-anhydroglucitol, FPG fasting plasma glucose, 2-hour PG 2-hour postload glucose, CI confidence interval



**Table 3** Crude and multivariable-adjusted ORs and 95 % CIs for the presence of carotid wall thickening according to quartiles of glycemic measures in subjects with glucose intolerance

	No. of cases/subjects	Crude OR (95 % CI)	P value	P for trend	Multivariable-adjusted OR (95 % CI)	P value	P for trend
<b>HbA<sub>1c</sub>, % (mmol/mol)</b>							
Q1, <5.4 (36)	99/243	1.00 (reference)		<0.001	1.00 (reference)		<0.001
Q2, 5.4–5.6 (36–38)	107/271	0.95 (0.67 to 1.35)	0.77		0.94 (0.64 to 1.37)	0.73	
Q3, 5.7–6.2 (39–44)	168/326	1.55 (1.11 to 2.16)	0.01		1.32 (0.91 to 1.92)	0.15	
Q4, ≥6.3 (45)	156/259	2.20 (1.54 to 3.15)	<0.001		1.96 (1.32 to 2.93)	<0.001	
<b>GA, %</b>							
Q1, <14.3	102/273	1.00 (reference)		<0.001	1.00 (reference)		<0.001
Q2, 14.3–15.3	114/272	1.21 (0.86 to 1.71)	0.28		1.23 (0.84 to 1.79)	0.29	
Q3, 15.4–17.1	146/279	1.84 (1.31 to 2.58)	<0.001		1.68 (1.15 to 2.45)	0.007	
Q4, ≥17.2	168/275	2.63 (1.87 to 3.72)	<0.001		2.12 (1.45 to 3.10)	<0.001	
<b>1,5-AG, µg/mL</b>							
Q1, <11.0	158/277	1.47 (1.05 to 2.06)	0.02	0.03	1.43 (0.99 to 2.07)	0.05	0.09
Q2, 11.0–16.9	120/273	0.87 (0.62 to 1.22)	0.41		0.87 (0.60 to 1.26)	0.47	
Q3, 17.0–22.9	121/273	0.88 (0.63 to 1.23)	0.46		0.97 (0.67 to 1.41)	0.88	
Q4, ≥23.0	131/276	1.00 (reference)			1.00 (reference)		
<b>FPG, mmol/L</b>							
Q1, <5.7	121/285	1.00 (reference)		<0.001	1.00 (reference)		<0.001
Q2, 5.7–6.1	120/279	1.02 (0.73 to 1.43)	0.89		0.86 (0.59 to 1.23)	0.40	
Q3, 6.2–6.8	123/258	1.24 (0.88 to 1.73)	0.22		1.26 (0.87 to 1.82)	0.23	
Q4, ≥6.9	166/277	2.03 (1.45 to 2.84)	<0.001		1.76 (1.21 to 2.56)	0.003	
<b>2-hour PG, mmol/L</b>							
Q1, <8.0	119/286	1.00 (reference)		<0.001	1.00 (reference)		0.14
Q2, 8.0–9.1	123/264	1.22 (0.87 to 1.72)	0.24		1.04 (0.72 to 1.50)	0.85	
Q3, 9.2–12.3	125/273	1.19 (0.85 to 1.66)	0.32		0.92 (0.64 to 1.32)	0.64	
Q4, ≥12.4	163/276	2.02 (1.45 to 2.83)	<0.001		1.41 (0.97 to 2.04)	0.07	

Multivariable adjustment was made for age, sex, hypertension, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body mass index, alcohol intake, smoking habits, regular exercise, and lipid-lowering medication. Because serum 1,5-AG levels are decreased in the presence of hyperglycemia, the highest quartile was used as the reference group for 1,5-AG

HbA<sub>1c</sub> hemoglobin A<sub>1c</sub>, GA glycated albumin, 1,5-AG 1,5-anhydroglucitol, FPG fasting plasma glucose, 2-hour PG 2-hour postload glucose, OR odds ratio, CI confidence interval

**Table 4** Comparison of the discriminative ability for detecting the presence of carotid wall thickening between the models with and without each glycemic measure among subjects with glucose intolerance

Model	Area under curve (95 % CI)	P value (vs. Model 1)
Model 1	0.719 (0.689 to 0.748)	reference
Model 1 + HbA <sub>1c</sub>	0.729 (0.699 to 0.758)	0.04
Model 1 + GA	0.728 (0.699 to 0.758)	0.04
Model 1 + 1,5-AG	0.721 (0.691 to 0.751)	0.27
Model 1 + FPG	0.724 (0.694 to 0.753)	0.19
Model 1 + 2-hour PG	0.721 (0.691 to 0.751)	0.39

Model 1 includes age, sex, hypertension, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body mass index, alcohol intake, smoking habits, regular exercise, and lipid-lowering medication  
HbA<sub>1c</sub> hemoglobin A<sub>1c</sub>, GA glycated albumin, 1,5-AG 1,5-anhydroglucitol, FPG fasting plasma glucose, 2-hour PG 2-hour postload glucose

that HbA<sub>1c</sub> levels were not independently predictive of CVD incidence and mortality [41, 42], our previous study revealed that elevated HbA<sub>1c</sub> levels were significantly associated with increased risk of the development of CVD [43]. Other prospective studies in Western populations have also shown significant positive associations between HbA<sub>1c</sub> levels and the risk of CVD [9, 44]. These findings suggest that a higher HbA<sub>1c</sub> level is a risk factor for sub-clinical and clinical CVD in Asian populations including Japanese as well as in Western populations.

Epidemiological studies of community-dwelling persons which investigated the association between GA levels and atherosclerosis have been scarce. Only a Japanese community-based study has reported that maximum carotid IMT increased in subjects with higher GA levels, although it did not fully take confounding factors into consideration [5]. In our study, the likelihood of

carotid wall thickening increased linearly with elevating GA levels among individuals with GI, and this association remained robust even after controlling for other cardiovascular risk factors. With regard to clinical studies, a recent case-cohort study in subjects with type 1 diabetes failed to demonstrate a significant influence of GA levels on CVD incidence and death [15]. Another study of patients with type 1 diabetes has also reported no significant association between GA/HbA<sub>1c</sub> ratio and carotid IMT [25]. However, other studies of patients on dialysis and those with type 2 diabetes have shown that higher GA levels were associated with the presence and progression of carotid atherosclerosis [20, 22, 24], the presence of coronary artery disease [18], impaired coronary collateral growth [23], and future CVD mortality [21] or hospitalization [19]. These findings, when taken together with our present results, indicate a close association between GA levels and the risk of atherosclerosis.

The present analysis demonstrated no clear association of 1,5-AG levels with maximum carotid IMT even in the crude analysis. Prior clinical studies also did not reveal significant differences in mean or maximum carotid IMT across 1,5-AG levels in patients with diabetes or hypertension [26, 27]. These findings imply that a weak association exists between 1,5-AG levels and carotid atherosclerosis. One of the possible reasons for this phenomenon is that the 1,5-AG level depends on the magnitude of glycosuria rather than plasma glucose levels [17], while other glycemic measures, including HbA<sub>1c</sub>, GA, FPG and 2-hour PG, directly reflect the degree of hyperglycemia. Another reason may be that 1,5-AG levels were affected by individual differences in the renal thresholds for glucose [45]. These explanations may account for the relatively weak association between 1,5-AG levels and carotid atherosclerosis in our study. On the other hand, with regard to clinical CVD events, one population-based cohort study in Japan has shown that lower 1,5-AG levels significantly increased the risk of developing CVD in men [28]. Since few studies have investigated the associations between 1,5-AG levels and the risk of CVD, further research is needed to clarify this issue.

In our study, higher FPG levels were independently related to carotid wall thickening in subjects with GI, but 2-hour PG levels were not. There have been conflicting data regarding the association between 2-hour PG levels and carotid atherosclerosis. At least 4 studies have shown that higher 2-hour PG levels were not a relevant factor for carotid atherosclerosis [3, 7, 11, 46], although others have found that elevated 2-hour PG levels were associated with increased carotid IMT [6, 47, 48]. Our previous prospective study showed that increased 2-hour PG levels were an independent risk factor for the occurrence of clinical CVD [49]. Thus, 2-hour PG levels

might be more associated with the advanced stages of atherosclerosis than the early stages. Further investigation is necessary to validate this hypothesis.

In the present study, adding HbA<sub>1c</sub> or GA to a model including known cardiovascular risk factors significantly increased the AUCs for the presence of carotid wall thickening in subjects with GI, while the addition of 1,5-AG, FPG, and 2-hour PG did not substantially increase them. These results suggest that HbA<sub>1c</sub> and GA provide greater improvement in discriminative ability for detecting carotid atherosclerosis than other glycemic measures. Advanced glycation end products (AGEs) are recognized as one of the important contributors to the pathogenesis of atherosclerosis in hyperglycemia [50]. It is also known that HbA<sub>1c</sub> and GA are non-enzymatically glycosylated proteins, and these two measures are regarded as precursors of AGEs [51, 52]. Thus, it is speculated that HbA<sub>1c</sub> and GA values are correlated with the amount of AGEs. These findings raise the possibility that AGEs may play a key role in the early stages of atherosclerosis. Furthermore, it has been reported that HbA<sub>1c</sub> had a strong correlation with elevated FPG levels, which are mainly caused by insulin resistance, while GA was closely related to higher postprandial glucose levels, which are attributed to reduced insulin secretion [53]. Considering this finding, higher HbA<sub>1c</sub> levels may indicate insulin resistance, while elevated GA levels are likely to reflect decreased insulin secretion, resulting in postprandial glucose excursion. Since insulin resistance [54], abdominal obesity [55, 56], and glucose excursion [57] were also found to play important roles in the pathogenesis of atherosclerosis, higher HbA<sub>1c</sub> and GA levels may contribute to increased carotid IMT through insulin resistance, glucose excursion as well as AGEs, and this might be the reason why measurements of HbA<sub>1c</sub> and GA improve the discriminative ability for the presence of carotid wall thickening. In addition, because HbA<sub>1c</sub> and GA measurements can be done without fasting or timed samples, these two measures are convenient and suitable for use in general practice. This advantage has implications for the identification and management of atherosclerosis in its early stages, and thus measurements of HbA<sub>1c</sub> and GA suggest to be useful in the assessment of atherosclerosis.

In our subjects with NGT, the crude ORs for the presence of carotid wall thickening increased with rising HbA<sub>1c</sub>, GA, FPG, and 2-hour PG levels, but these associations were markedly attenuated after adjustment for other covariates. These findings suggest that higher glucose levels within normal range may not contribute independently to increased carotid IMT. Since other known cardiovascular risk factors, such as aging, hypertension, and dyslipidemia, tended to accumulate at the higher levels of HbA<sub>1c</sub>, GA, FPG, and 2-hour PG in our

subjects with NGT (data not shown), individuals with elevations of these glycemic measures would seem to have higher carotid IMT through the mediation of other coexisting risk factors.

The strengths of our study include the population-based design and high participation rate. We also made a comparison of the five glycemic measures, which was not done in prior studies. However, some limitations should be mentioned. First, the cross-sectional study design limits the interpretation of causality between levels of glycemic measures and carotid atherosclerosis. Since the usefulness of glycemic measures in the assessment of atherosclerosis would ideally be evaluated in studies that examine the association between measures of glycemia and incident atherosclerotic disease, further prospective investigation is expected. Second, each glycemic measure was based on a single measurement, as was the case in most other epidemiological studies. This limitation may have resulted in misclassification of study subjects into different categories, and such misclassification could have weakened the association found in our study, biasing the results toward the null hypothesis. Third, the serum GA and 1,5-AG levels were measured after being stored at -80 °C for 5 years. In this context, however, we should note that the stability of GA and 1,5-AG measurements in frozen stored serum sample has been confirmed [58, 59]. Fourth, several laboratory technicians took ultrasound images of carotid artery without assessment of inter-observer variability. However, they were specially trained to use a standardized technique, and the IMT was measured automatically using a computer-assisted measurement system. Thus, we believe that this limitation is not likely to invalidate the findings observed in the present study. Fifth, CKD may cause a change in 1,5-AG levels through alterations in renal hemodynamics. However, the sensitivity analyses excluding subjects with CKD did not make any material difference in the findings on the association between 1,5-AG levels and carotid IMT (data not shown). Lastly, sample size of our study was relatively small to perform analyses separately in subjects with diabetes and prediabetes. Further studies with larger sample size are required to elucidate this issue.

## Conclusions

The present analysis showed that, in a Japanese population with GI, elevated HbA<sub>1c</sub>, GA, and FPG levels were significantly associated with increased carotid IMT, and that the ability of HbA<sub>1c</sub> and GA to detect carotid wall thickening was superior to that of 1,5-AG, FPG, and 2-hour PG, suggesting that measurements of HbA<sub>1c</sub> and GA are useful as markers of early atherosclerosis. Further prospective studies are needed to verify these findings.

## Additional file

**Additional file 1: Figure S1.** Multivariable-adjusted geometric average of maximum intima-media thickness according to quartiles of each glycemic measure in subjects with normal glucose tolerance. **Table S1:** The associations of 1 SD increment in HbA<sub>1c</sub>, GA, FPG, and 2-hour PG, and 1 SD decrement in 1,5-AG with the maximum intima-media thickness in subjects with normal glucose tolerance. **Table S2:** Crude and multivariable-adjusted odds ratios and 95 % confidence intervals for the presence of carotid wall thickening according to quartiles of glycemic measures in subjects with normal glucose tolerance. **Figure S2A and S2B:** Multivariable-adjusted odds ratios and their 95 % confidence intervals for the presence of carotid wall thickening per 1 SD increment in HbA<sub>1c</sub>, GA, FPG, and 2-hour PG, and 1 SD decrement in 1,5-AG according to the subgroups of sex, age, smoking habits, insulin resistance, hypertension, and hyper-LDL cholesterolemia in subjects with glucose intolerance.

## Abbreviations

HbA<sub>1c</sub>: Hemoglobin A<sub>1c</sub>; IMT: Intima-media thickness; GA: Glycated albumin; 1,5-AG: 1,5-anhydroglucitol; FPG: Fasting plasma glucose; PG: Postload glucose; CVD: Cardiovascular disease; OGTT: Oral glucose tolerance test; NGT: Normal glucose tolerance; GI: Glucose intolerance; NGSP: National Glycohemoglobin Standardization Program; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; eGFR: Estimated glomerular filtration rate; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; CKD: Chronic kidney disease; BMI: Body mass index; OR: Odds ratio; CI: Confidence interval; AUC: Area under the receiver operating characteristic curve; AGE: Advanced glycation end product.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

NM contributed to the study concept and design, data collection, data analysis, data interpretation, and drafting of the manuscript. TN, JH, YH, FI, and MF contributed to the data collection and data interpretation. TH and DK measured the samples and contributed to the data interpretation. MK, UN, and TK contributed to the data interpretation and reviewed/edited the manuscript. YK contributed to the data collection, data interpretation, drafting of the manuscript, obtained funding, and study supervision. All authors provided critical review of the draft and approved the final version.

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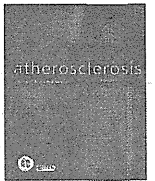
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## Hematocrit and the risk of cardiovascular disease in a Japanese community: The Hisayama Study



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### ABSTRACT

**Objectives:** The association between hematocrit levels and the risk of cardiovascular disease (CVD) has been reported inconsistently. We aimed to investigate the association of hematocrit levels with the development of stroke and coronary heart disease (CHD) in a general Japanese population.

**Methods:** A total of 2585 community-dwelling Japanese individuals aged 40 years or older in 1988 were followed up for 19 years. These subjects were divided into four groups according to the sex-specific quartiles of hematocrit at baseline.

**Results:** During the follow-up, 301 subjects developed stroke (210 ischemic and 91 hemorrhagic) and 187 developed CHD. The risk of ischemic stroke was higher in both the lowest (Q1: men,  $\leq 44.7\%$ ; women,  $\leq 39.3\%$ ) and the highest (Q4: men,  $\geq 49.7\%$ ; women,  $\geq 43.8\%$ ) quartiles than in the third quartile (Q3: men, 47.1%–49.6%; women, 41.7%–43.7%) used as a reference (multivariable-adjusted hazard ratios [95% confidence intervals]: Q1, 1.55 [0.99–2.43]; Q2, 1.44 [0.93–2.23]; Q3, 1.00; and Q4, 1.62 [1.06–2.50];  $P = 0.86$  for trend). In contrast, hematocrit levels and the risk of hemorrhagic stroke showed a linear inverse association (Q1, 1.91 [1.03–3.54]; Q2, 1.26 [0.68–2.34]; Q3, 1.00; and Q4, 0.81 [0.41–1.61];  $P = 0.009$  for trend). The risk of CHD increased significantly in Q4 (Q1, 1.13 [0.71–1.80]; Q2, 1.08 [0.69–1.71]; Q3, 1.00; and Q4, 1.60 [1.04–2.46];  $P = 0.13$  for trend).

**Conclusions:** Our findings suggest that both elevated and decreased hematocrit levels are associated with an increased risk of CVD, but the influence of hematocrit is different among subtypes of CVD.

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Hematocrit, the proportion of the total blood volume occupied by red blood cells, is a major determinant of blood viscosity and plays an important role in regulating blood flow [1,2]. Although several community-based surveys have examined the association between hematocrit levels and risk of cardiovascular disease (CVD), the findings have been inconsistent [3–11]. We previously reported that a lower hematocrit level was associated with higher incidence of ischemic stroke in women based on the follow-up survey of a general Japanese population over the period from 1965 to 1981 [3]. However, since the hematocrit level in Japanese has increased greatly over the following several decades, the influence of

hematocrit on the development of stroke might have changed. Since higher hematocrit was reported to be a risk factor for ischemic stroke in Western populations [4–6], we hypothesized that not only lower level but also higher level of hematocrit may be associated with the risk of ischemic stroke in the recent Japanese population. In addition, the association between hematocrit levels and coronary heart disease (CHD) has been investigated mainly in Western populations [5–11]. Therefore, a prospective study on the association between hematocrit and stroke or CHD in a more recent Japanese population would be very helpful for understanding hematocrit as a risk factor for CVD and for reducing the burden of CVD in Japan and other Asian countries.

The purpose of the present study was thus to investigate the association of hematocrit levels with the development of CVD, including ischemic and hemorrhagic stroke and CHD, in a recent

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# Midlife and Late-Life Smoking and Risk of Dementia in the Community: The Hisayama Study

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**OBJECTIVES:** To clarify the association between midlife and late-life smoking and risk of dementia.

**DESIGN:** Prospective cohort study.

**SETTING:** The Hisayama Study, Japan.

**PARTICIPANTS:** Japanese community-dwellers without dementia aged 65 to 84 (mean age 72) followed for 17 years (1988–2005) (N = 754), 619 of whom had participated in a health examination conducted in 1973–74 (mean age, 57) and were included in the midlife analysis.

**MEASUREMENTS:** The risk estimates of smoking status on the development of dementia were computed using a Cox proportional hazards model.

**RESULTS:** During follow-up, 252 subjects developed all-cause dementia; 143 had Alzheimer's disease (AD), and 76 had vascular dementia (VaD). In late life, the multivariable-adjusted risk of all-cause dementia was significantly greater in current smokers than in never smokers; similar associations were seen for all-cause dementia, AD, and VaD in midlife current smokers. Meanwhile, no significant association was observed between past smoking and risk of any type of dementia in late or midlife. Multivariable analysis showed that smokers in midlife and late life had significantly greater risks than lifelong nonsmokers of all-cause dementia (adjusted hazard ratio (aHR) = 2.28, 95% confidence interval (CI) = 1.49–3.49), AD (aHR = 1.98, 95% CI = 1.09–3.61), and VaD (aHR = 2.88, 95% CI = 1.34–6.20). Such associations were not observed for midlife smokers who quit smoking in late life.

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**CONCLUSION:** Persistent smoking from mid- to late life is a significant risk factor for dementia and its subtypes in the general Japanese population. *J Am Geriatr Soc* 63:2332–2339, 2015.

**Key words:** dementia; Alzheimer's disease; vascular dementia; cohort studies; smoking

Dementia is a major cause of disability and premature death in elderly adults.<sup>1</sup> Because the number of people with dementia has been increasing worldwide,<sup>1</sup> the burden of dementia has become a serious social, medical, and economic problem, but the causes of dementia, especially Alzheimer's disease (AD), remain unclear, and no disease-modifying therapies have been established. Thus, there is an urgent need to identify modifiable risk factors for dementia. Smoking has been reported to increase risk of stroke,<sup>2</sup> diabetes mellitus,<sup>3</sup> and hypertension,<sup>4</sup> all of which are associated with the development of dementia,<sup>5</sup> but data regarding its direct association with dementia, especially AD, have been inconsistent.<sup>6–21</sup> Several early case-control studies have suggested that late-life smoking might have an inverse influence on dementia,<sup>6–9</sup> whereas some population-based prospective studies have reported that late-life smoking is a risk factor for the development of AD,<sup>10–14</sup> but most of the prospective studies included follow-up periods of less than 10 years,<sup>10–13</sup> and there have been few population-based prospective studies examining the association between midlife smoking and risk of dementia.<sup>15,16</sup> Furthermore, there is a possibility that smoking increases the risk of vascular dementia (VaD) by mediating cerebrovascular diseases, but few prospective studies have examined this.<sup>12,14–16</sup> The purpose of this study was to investigate the association between smoking in mid- and late life and the development of dementia and its subtypes in a prospective cohort study of an elderly Japanese population.

## Original Article

## Angiotensin I-Converting Enzyme Gene Polymorphism Enhances the Effect of Hypercholesterolemia on the Risk of Coronary Heart Disease in a General Japanese Population: The Hisayama Study

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**Aim:** The angiotensin I-converting enzyme (ACE) insertion (I)/deletion (D) polymorphism has been reported to be implicated in susceptibility to coronary heart disease (CHD). However, this association remains inconclusive. The purpose of this study was to clarify the association between the I/D polymorphism of the ACE gene and the development of CHD in a Japanese general population and investigate whether the effects of traditional risk factors on the risk of CHD are heterogeneous among ACE genotypes.

**Methods:** The subjects included 2,125 community-dwelling individuals 40 years of age or older without cardiovascular disease for whom genetic information was available. All patients were prospectively followed for 19 years, and the association between the ACE polymorphism and the incidence of CHD was examined based on the interactions with traditional risk factors, such as hypercholesterolemia, hypertension, diabetes and smoking.

**Results:** A total of 161 CHD events occurred during the follow-up period. The age- and sex-adjusted incidence of CHD was not significantly different among the genotypes (5.8, 5.2, and 6.9 per 1,000 person-years for genotypes II, ID and DD, respectively). In a stratified analysis, however, the ACE DD genotype was found to significantly accelerate the risk of developing CHD by hypercholesterolemia (hazard ratio [HR]=4.50, 95% confidence interval=2.02-10.04 for hypercholesterolemia with the DD genotype; HR=1.48, 95% CI=1.04-2.12 for hypercholesterolemia with the ID+II genotype; P for interaction=0.01), even after adjusting for other confounding factors, whereas no such associations were observed for hypertension, diabetes or smoking.

**Conclusions:** The current findings suggest that the ACE DD genotype enhances the effect of hypercholesterolemia on the development of CHD in the general Japanese population.

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**Key words:** ACE polymorphism, Coronary heart disease, Cohort study, Hypercholesterolemia

### Introduction

Coronary heart disease (CHD) is a major cause of morbidity and mortality in industrialized countries<sup>1</sup>. Accumulated evidence has identified a number

of risk factors, including hypercholesterolemia, hypertension, diabetes and smoking, for the development of CHD<sup>2-4</sup>. The renin-angiotensin system has also been acknowledged to be involved in the pathogenetic mechanisms of CHD. The plasma activity of angio-



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Trajectories of cognitive function in dementia-free subjects: A RERF Adult Health Study

Michiko Yamada, Reid D. Landes, Yasuyo Mimori, Yoshito Nagano, Hideo Sasaki

ABSTRACT

**Objectives:** To investigate associations between age, sex, education, and birth cohort and global cognitive decline among a population that would most likely not progress to dementia.

**Methods:** A total of 1,538 dementia-free subjects aged 60 to 80 in 1992 were followed up through 2011 without dementia occurrence. We assessed cognitive function using the Cognitive Ability Screening Instrument (CASI). Using stepwise-like model selection procedure, we built mixed-effects models for initial cognition and longitudinal cognition.

**Results:** Initial CASI scores for younger age and more years of formal education were higher than those for older and less education. Sex did not show a significant effect. In the longitudinal analysis, cognitive decline became more rapid with increasing age. Sex and education did not modify the degree of deterioration with age. CASI scores were higher for younger cohorts and men due to differences in education levels.

**Conclusion:** Among dementia-free subjects, age is an important predictor of cognitive function level and cognitive decline. Education level affects cognitive function level, but did not affect cognitive decline. The results have implications not only for elucidation of the aging process, but also for reference in dementia screening.

Radiation effects on cognitive function among atomic bomb survivors exposed at or after adolescence

Michiko Yamada, Reid D Landes, Yasuyo Mimori, Yoshito Nagano, Hideo Sasaki

ABSTRACT

**Background:** The objective of this study was to investigate radiation effects on longitudinal pre-dementia cognitive decline among participants who developed dementia as well as on those who did not develop dementia during follow-up.

**Methods:** Measuring cognitive function with the Cognitive Abilities Screening Instrument (CASI) approximately every 2 years, we followed 1,844 atomic bomb survivors participating in the Adult Health Study of the Radiation Effects Research Foundation from 1992 to 2011. Participants were adolescents or older when exposed to between 0 and 4 Gy. About 15% and 40% of participants were exposed to  $\geq 1$  Gy and  $< 5$ mGy, respectively. At study start, participants were dementia-free and between 60–80 years-old. Three-quarters of the participants returned after baseline, averaging 8.4 years of follow-up. During follow-up, 313 developed dementia. We used cognitive scores before dementia onset for analysis and a mixed-effects model to estimate radiation effects on longitudinal change of cognition, adjusting for dementia occurrence, age, sex, and education.

**Results:** Cognition level was significantly associated with age, education, and dementia occurrence, but not with radiation dose or sex. Cognitive decline accelerated with increasing age, especially among participants who developed dementia. Neither radiation nor education was significantly associated with the degree of deterioration with age. Radiation did not modify the different cognitive decline by dementia occurrence.

**Conclusion:** Radiation did not significantly affect cognition among atomic bomb survivors exposed at or after adolescence.

## ORIGINAL ARTICLE

# The combination of abdominal obesity and high-sensitivity C-reactive protein predicts new-onset hypertension in the general Japanese population: the Tanno–Sobetsu study

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The aim of this study was to examine whether the combination of abdominal obesity and high serum levels of high-sensitivity C-reactive protein (hsCRP) improves the prediction of new-onset hypertension in the general Japanese population. Participants in the Tanno–Sobetsu study, a prospective cohort study, were enrolled. Of 1516 subjects aged 30 years or older in 2002, those with hypertension or abnormal hsCRP levels were excluded, and the remaining 705 subjects were included in the present analyses. Abdominal obesity (AO) and high hsCRP levels were defined by the Japanese criteria of waist circumference and median hsCRP values, respectively. Subjects were followed up on for a maximum of 4.5 years, and the hazard ratio (HR) for new-onset hypertension was calculated using the Cox proportional hazard model. The HR for new-onset hypertension after adjustment for age, sex, fasting plasma glucose, alcohol intake, smoking, treatment for dyslipidemia and high normal blood pressure at baseline was significantly higher in the group with AO and high hsCRP (HR = 1.44, 95% CI: 1.00–2.07) compared with the reference group, a group with no AO and low hsCRP; AO alone or high hsCRP alone was not associated with a significant increase in the HR. Similar trends for the increase in the HR by AO and high hsCRP were observed in separate analyses of men and women, although the differences did not reach statistical significance. Co-presence of AO and a high level of hsCRP is associated with a high risk for new-onset hypertension in the general population.

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**Keywords:** abdominal obesity; general population; high-sensitivity CRP; incidence of hypertension

## INTRODUCTION

In a report by the World Health Organization, hypertension is ranked first among the important risk factors associated with mortality and fifth among the risk factors of disability-adjusted life years.<sup>1</sup> A recent study on the risk factors for noncommunicable diseases confirmed that hypertension is a major risk factor for adult mortality, as is smoking.<sup>2</sup> Hypertension therapy has greatly advanced in the past four decades, and the prognosis of hypertension has improved. In addition, the importance of hypertension prevention has been recognized in society and by several organizations, including the Japanese Society of Hypertension,<sup>3</sup> which have formulated lifestyle modifications for preventing hypertension. Lifestyle modifications for hypertension prevention (that is, salt and alcohol restrictions, control of obesity, increase in physical activity, diet rich in fruits and vegetables and low fat intake) are beneficial and recommended for the general population. On the other hand, stratification of hypertension risks in normotensive subjects is important for the efficient use of social resources for

education and lifestyle guidance. However, how to stratify hypertension risks in normotensive adults has not yet been established except for the fact that blood pressure at high normal blood pressure levels poses a great risk of new-onset hypertension.<sup>4</sup>

We hypothesized that the risk of hypertension in normotensives is better predicted by a combination of multiple risk factors, and we selected abdominal obesity and elevated levels of serum C-reactive protein (CRP) as indices for the combination. Obesity is an established risk factor of hypertension and cardiac diseases, and earlier studies have demonstrated a significant association of abdominal obesity (AO) with subsequent development of hypertension in various ethnic groups.<sup>5–10</sup> Conversely, weight reduction has been shown to reduce blood pressure in hypertensive subjects.<sup>11</sup> Chronic low-grade inflammation has also been shown to be involved in blood pressure elevation and atherosclerosis.<sup>12–25</sup> Inflammatory cytokines and upregulated production of reactive oxygen species induce endothelial dysfunction and vascular remodeling.<sup>26,27</sup> CRP is produced in the liver in response

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## Combination of type 2 diabetes and smoking increases total cancer mortality in Japanese men using competing risk analysis: the Tanno-Sobetsu study

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### Abstract

**Aims** We assessed the impact of the combination of type 2 diabetes (T2DM) and smoking on total cancer mortality using cohort data of a general Japanese population.

**Methods** Of 1908 residents who received health checkups in two towns in 1994, 794 males were included as study participants. Smoking status was defined as three categories: never smoker, ex-smoker and current smoker. Individuals with T2DM were defined as individuals with fasting plasma glucose  $\geq 7.0$  mmol/l and/or receiving medication for T2DM. Participants were divided into six groups according to T2DM and smoking status: non-DM (NDM) and never smoker, NDM and ex-smoker, NDM and current smoker, DM and never smoker, DM and ex-smoker, and DM and current smoker groups. All participants were followed up for a maximum of 13 years. We calculated the hazard ratio (HR) using Cox's proportional hazard model and subhazard ratio (SHR) using competing risk regression analyses in each group.

**Results** During the follow-up period, there were 169 all-cause deaths (62 cancer deaths and 40 CVD deaths). When using Cox regression analysis, HRs were higher in

the ex-smoker and current smoker groups than in the never-smoker group, and HRs were also higher in the DM groups than in the NDM groups. When using competing risk analysis, SHRs were almost the same as the HRs of Cox regression analysis (DM and ex-smoker 6.06, DM and current smoker 10.12).

**Conclusions** The combination of T2DM and smoking is a strong risk factor for total cancer mortality in Japanese men.

**Keywords** Type 2 diabetes · Smoking · Cancer mortality · Competing risk analysis

### Introduction

Type 2 diabetes mellitus has recently been shown to be associated with cancer incidence and mortality [1–8]. Hyperinsulinemia accompanied by insulin resistance, hyperglycemia and chronic inflammation are known to be mechanisms of oncogenesis. Another reason why type 2 diabetes is related to cancer is that individuals with type 2 diabetes are likely to have common cancer risk factors such as obesity, smoking and alcohol drinking. Cigarette smoking is a strong risk factor for various types of cancer [9]. However, the effect of the combination of type 2 diabetes and smoking on cancer mortality is still unclear.

Both type 2 diabetes and smoking are also risk factors of cardiovascular disease [10], and cardiovascular death may preclude the possibility of cancer death due to type 2 diabetes and smoking in later years. Therefore, when assessing the effects of these two risk factors on cancer mortality, cardiovascular death should be considered as a competing risk. In such cases, a competing risk analysis such as the Fine and Gray model is recommended rather than the

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