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| **Full title** | Association of hematological parameters with insulin resistance, insulin sensitivity, and asymptomatic cerebrovascular damage: the J-SHIP Toon Health Study |

| **Short title** | Hematological parameters and insulin homeostasis |

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ABSTRACT

Background Elevated hematocrit levels have been suggested to be an independent determinant of insulin resistance and type 2 diabetes. To clarify the diagnostic significance of hematocrit level, we investigated the association with hemodynamic profiles, insulin resistance and insulin sensitivity, arterial properties, and asymptomatic cerebrovascular damage in a general Japanese population.

Methods This study included 1,978 participants from two independent cohorts. Insulin sensitivity was assessed by the oral 75 g glucose tolerance test. Carotid ultrasonography was performed to evaluate atherosclerosis and wall shear stress. Periventricular hyperintensity and lacunar infarction were assessed by brain magnetic resonance imaging.

Results Hematocrit quartile showed a stepwise association with insulin sensitivity (Q1: 2.2 ± 0.7, Q2: 2.0 ± 0.7, Q3: 1.9 ± 0.7, Q4:1.8 ± 0.6, $p<0.001$) and insulin resistance (1.0 ± 0.6, 1.2 ± 0.7, 1.3 ± 0.8, 1.5 ± 1.0, $p<0.001$). Multiple linear regression analysis adjusted for possible covariates identified hematocrit as an independent determinant of insulin sensitivity (β=-0.074, $p=0.019$) and insulin resistance (β=0.115, $p<0.001$). However, this association was lost after further adjustment for visceral fat area and plasma alanine aminotransferase level. Further, no significant association was observed between hematocrit and carotid intima-media thickness ($p=0.306$) whereas wall shear stress was inversely associated with the carotid atherosclerosis (r=-0.250, $p<0.001$). In contrast, a low hematocrit level was independently associated with periventricular hyperintensity (odds ratio 0.87 (95% CI 0.80-0.95), $p=0.001$).

Conclusion Hematocirt was positively associated with insulin resistance and insulin sensitivity. This association was epiphenomenon of visceral and hepatic adiposity. Conversely, low hematocrit was a significant risk factor for periventricular hyperintensity independent of insulin resistance.

INTRODUCTION

Insulin resistance is a key factor underlying the relationship between abdominal obesity and metabolic diseases. It was well established that insulin resistance predicts the future development of type 2 diabetes [1], cardiovascular disease [2, 3], as well as mortality [4, 5]. Several plasma markers, namely inflammatory cytokines [6], C-reactive protein [7], and adiponectin [8] among others have been associated with insulin resistance. As for hematological parameters, white blood cell (WBC) count is well known to reflect inflammatory status and insulin resistance [9].

A number of studies have reported that elevated hematocrit (Hct) also reflects insulin resistance and type 2 diabetes [10-12]. Tulloch-Reid et al. [10] reported that elevated Hct is associated with a higher risk of developing type 2 diabetes in Pima Indians, which was possibly mediated via an association with insulin resistance. Hanley et al. [12] reported highly significant associations of Hct with both insulin resistance and β-cell dysfunction in non-diabetic subjects. Further, prognostic significance of increased Hct levels on coronary heart disease mortality was shown in a longitudinal study [13]. Since Hct is a major determinant of blood viscosity [14], increased viscosity and the consequent limited delivery of glucose and insulin to peripheral organs, i.e. so-called flow-related insulin resistance, has been postulated as an underlying mechanism implicating the relationship between Hct and insulin resistance [15]. Alternatively, elevated Hct levels in persons with hyperinsulinemia might also result from reduced plasma volume caused by increased insulin stimuli. Insulin increases muscle capillary blood flow and transcapillary albumin transport [16], which in turn causes decreased plasma volume and consequent high Hct.

To clarify whether an increase in Hct level in the general population reflects insulin resistance, we conducted a cross-sectional study in Japanese. Since insulin resistance is a major risk factor for arteriosclerotic end-organ damage, we also investigated possible associations between Hct levels and arterial stiffness, as well as asymptomatic

cerebrovascular damage.

METHODS

Study subjects: the anti-aging center Study

The study subjects consisted of 952 apparently healthy middle-aged to elderly persons. All subjects were consecutive participants in the medical check-up program at Ehime University Hospital Anti-aging Center (AAC) during February 2006 to March 2011. Participants who had a history of symptomatic stroke, muscle-skeletal systems disorders, and definitive dementia were excluded from the analysis. This check-up program is specifically designed to evaluate aging-related disorders, including atherosclerosis, cardiovascular disease, physical function, and mild cognitive impairment. All clinical data used in this study were obtained through the check-up process. This cross-sectional investigation was carried out as part of the Shimanami Health Promoting Program (J-SHIPP study), a longitudinal study evaluating factors related to cardiovascular disease, dementia, and death [17]. This series of studies was approved by the ethics committee of Ehime University Graduate School of Medicine. All study subjects provided informed consent.

Study subjects: the Toon Health Study

The present study was conducted as part of the Toon Health Study (THS), an ongoing longitudinal epidemiological study begun in 2009 in the general population living in Toon City, Ehime Prefecture, Japan [18]. The goal of this study is to identify novel environmental and genetic risk factors related to incident diabetes and cardiovascular disease. Toon City is a largely rural area located on Shikoku Island in southern Japan with a population of approximately 35,000. Subjects were recruited from residents aged 40-79 years. Of the 1,109 participants enrolled in the study from 2009 to 2010, 1,026 who were not taking antihyperglycemic agents were ultimately included in this analysis, as people receiving

hyperglycemic treatment are unable to undergo the oral 75 g glucose tolerance test (OGTT). Subjects with abnormal plasma alanine aminotransferase (ALT) levels of more than 100 IU/l were also excluded from the analysis. Basic plasma markers, including hematological parameters, were analyzed in a commercial laboratory. The THS was approved by the ethics committee of Ehime University Graduate School of Medicine, and written informed consent was obtained from all participants.

OGTT

After fasting overnight, all subjects participated in the THS took 75 g of glucose dissolved in 225 ml of water. Venous blood samples were obtained at 0, 60, and 120 min for measurement of plasma glucose and insulin. The revised version of homeostasis model assessment index for insulin resistance (HOMA2-IR) was calculated from fasting glucose (mmol/L) and insulin (pmol/l) by using a HOMA Calculator (http://www.dtu.ox.ac.uk/homacalculator/index.php) as an index of insulin resistance [19]. Insulin sensitivity index (ISI) was calculated using Gutt's equation [20]:

$$ISI = [75{,}000 \text{ mg} + (glucose_0 - glucose_{120}) \times 0.19 \times \text{body weight} / 120 \text{ min}] /$$

$$[(glucose_0 + glucose_{120}) / 2] / \log [(insulin_0 + insulin_{120}) / 2].$$

Plasma glucose and insulin were determined by standard laboratory methods.

Measurement of abdominal visceral fat area

Visceral fat area (VFA) (cm^2) was measured from a computed tomography (CT) image (LightSpeed VCT; GE Healthcare, Tokyo, Japan) at the level of the umbilicus. CT images were obtained with a minimal slice width of 5 mm. Connected voxels with the CT attenuation range of -150 to -50 Hounsfield units were calculated as fat area. CT images were analyzed using OsiriX software [21].

Carotid ultrasonography

A series of ultrasonographic scans of the carotid artery was performed using an echotomographic system (SSD-3500SV or α10; Aloka Co., Ltd., Tokyo, Japan) with an electrical linear transducer (midfrequency 7.5 MHz). The common carotid artery in the neck was visualized from three longitudinal projections, namely the anterior-oblique, lateral, and posterior-oblique. In each image, the IMT of the far wall was measured at three points, 1 cm proximal to the bulb and ±1 cm distant from this site. The average of all nine values was used as the representative value. No measurements were taken at the level of a discrete plaque.

Two dimensional guided M-mode tracings of the bilateral carotid artery at a point 2 cm proximal to the bulb were recorded with an electrocardiogram, and the internal diameters of the artery at end-diastole and peak-systole were measured in three cycles and averaged. Axial resolution of the ultrasonography system was 0.1mm.

Doppler evaluation was performed on the right common carotid artery in the anterior projection. Under guidance by color flow mapping, blood flow velocity was detected at the center of the vessel. The angle between the ultrasound beam and the longitudinal vessel axis was kept between 45° and 55°. The peak-systolic and end-diastolic velocities were measured at three cardiac cycles.

Hemodynamic analysis

Whole blood viscosity (η) at 208 sec^{-1} was estimated by a previously validated formula [22] which takes account of Hct and plasma proteins:

$$\eta = 0.12 \times Hct\ (\%) + 0.17 \times total\ protein\ (mg/dl) - 2.07$$

Wall shear stress at peak-systole and end-diastole was calculated from the viscosity, carotid diameter (D) and carotid blood flow velocity (V) according to the following formula [23]:

$$wall\ shear\ stress\ (dyne/cm^2) = 4 \times \eta\ (poise) \times V\ (cm/sec.)\ /\ D\ (cm)$$

Assessment of asymptomatic cerebrovascular damage

Lacunar infarction was defined as areas of low signal intensity (3- to 15-mm diameter) on T1-weighted images and of high intensity on T2-weighted and FLAIR images. PVH were defined as white matter hyperintensities depicted on T2-weighted and FLAIR images in contact with the ventricular wall. PVH was further classified into five grades, namely grade 0, absent or only a "rim"; grade 1, limited lesion-like "caps"; grade 2, irregular "halo"; grade 3, irregular margins and extension into the deep white matter; and grade 4, extension into the deep white matter and subcortical portion. Imaging analysis was performed by two neurologists without clinical information on the subject.

Measurement of arterial stiffness

Brachial-to-ankle pulse wave velocity (baPWV) was measured as an index of arterial stiffness. To measure baPWV, cuffs were applied to both brachia and ankles, and all blood pressures (BPs) were measured simultaneously by the cuff-oscillometric method (BP-203RPEII (form PWV/ABI), Omron Healthcare, Co., Ltd., Kyoto, Japan). The pulse volume waveforms were also recorded simultaneously using a plethysmographic sensor connected to the cuffs. Brachial-to-ankle pulse wave velocity (baPWV) was calculated from the time interval between the wave fronts of the brachial and ankle waveforms, and the path length from the brachial to ankle. All measurements were performed in the supine position after at least 5 min rest. A brief explanation of this device as well as the validity and reproducibility of its measurements have been provided elsewhere [24].

Statistical analysis

Differences in numeric variables were assessed by analysis of variance. Frequency differences were analyzed by a chi-squared test. Factors independently associated with insulin resistance were assessed by a multiple linear regression analysis. All statistical analyses were performed

using a commercially available statistical software package (JMP 8.0 SAS Institute, Cary, NC). P values less than 0.05 was considered statistically significant.

RESULTS

Association of Hct level with insulin sensitivity and insulin resistance

Clinical characteristics of the study subjects are shown in Table 1. Hematological parameters, namely Hct and WBC count, were significantly higher in males (Supplemental Table 1, Supplemental Table 2). Insulin sensitivity in the THS population was lower in male than female subjects.

Associations of Hct quartile with ISI and HOMA-IR are shown in Figure 1. In this analysis, study subjects were divided into quartiles by sex and then combined to avoid potential sex differences. Significant positive associations were seen between Hct quartile and HOMA2-IR. Hct was also significantly associated with ISI, albeit with the opposite direction to HOMA2-IR. Multiple linear regression analysis adjusted for possible confounding factors identified Hct as an independent determinant of HOMA2-IR and ISI (Table 2). The association between Hct and HOMA2-IR was also independent of WBC. However, the association was lost after further adjustment for visceral fat area and plasma ALT level (Table 2, model 2), possibly due to the strong correlation between Hct level and VFA ($r=0.342$, $p<0.001$), as well as ALT ($r=0.314$, $p<0.001$). In this statistical model, co-linearity between body mass index (BMI) and VFA was statistically negligible (variance inflation factor: BMI=2.212, VFAT=2.813).

Associations of Hct level with hemodynamic parameters and arterial properties

Hemodynamic characteristics of the AAC subjects are summarized in Supplemental Table 3. Differences in hemodynamic parameters among the Hct quartile are shown in Table 3. Wall shear stress calculated by viscosity, blood velocity, and arterial diameter was showed a

positive association with Hct. However, no significant association was observed between Hct and arterial parameters, namely baPWV and carotid IMT, whereas these arterial parameters were inverse correlated with wall shear stress (baPWV: r=-0.250, p<0.001, carotid IMT: r=-0.180, p<0.001).

Association of Hct level with cerebrovascular damage

The number of subjects in each PVH grade is shown in Table 1. Subjects with grade 2 or higher were considered as having PVH. Frequency differences in PVH and lacunar infarction by Hct quartile are shown in Figure 2. Subjects in the lowest quartile had a higher prevalence of PVH but not of lacunar infarction. Although Q1 subjects were significantly older and had higher BNP levels (Supplemental Table 4), logistic regression analysis identified low Hct level as an independent determinant of PVH (p=0.001, odds 0.87 (95% C.I. 0.80-0.95) after adjustment for age, sex, BMI, hypertension, HOMA2-IR, baPWV and WBC count. Conversely, subjects with PVH showed significantly lower Hct levels and red blood cell count (RBC) (Supplemental figure) even in the sex-separated analysis (Hct: male, 43.2±4.3 vs. 45.1±3.1%, p=0.001, female, 39.9±3.0 vs. 41.1±2.7%, p=0.003; RBC: 4.6±0.5 vs. 4.8±0.4 $x10^4$/μl, p=0.001, female, 4.3±0.4 vs. 4.5±0.3 $x10^4$/μl, p=0.009) whereas no differences were observed with mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels. In contrast, WBC quartile was significantly associated with the frequency of lacunar infarction (Figure 2). However, this association was lost after adjustment for age, sex, and BMI (p=0.149).

DISCUSSION

In the present study, we showed that Hct was significantly associated with HOMA2-IR in two independent general populations. Further, we showed inverse association between Hct and ISI. However, this association was lost after adjustment of visceral fat area and ALT. Since

adiposity was associated with both Hct level, and insulin resistance and insulin sensitivity, adiposity may be an underlying factor for the associations observed between increased Hct levels and worsened insulin profile. The association between Hct and HOMA2-IR might be epiphenomenon of visceral and hepatic adiposity.

A reduction in blood flow consequent to increased Hct levels have been suggested to exacerbate insulin resistance by reducing glucose and insulin delivery to skeletal muscle [15]. However, elevated Hct concomitantly increased wall shear stress. Higher shear stress enhances nitric oxide (NO) production at the endothelium, and NO-dependent vasodilation activates insulin signaling and glucose uptake at the periphery. Actually, we observed inverse association between wall shear stress and baPWV, as well as carotid IMT. Higher Hct might therefore act to improve insulin resistance. Given these physiological characteristics, as well as our result that adiposity was a confounding factor for the association between Hct and HOMA2-IR, the increase in Hct in subjects with insulin resistance may be a result, rather than a cause, of increased plasma insulin levels. The lack of association of Hct quartile with baPWV or carotid IMT supports this conclusion.

Why Hct levels in subjects with increased insulin resistance were higher than that in normal control? Insulin reduces plasma volume by increasing the transcapillary escape of albumin [16]. This non-adrenergic-mediated plasma volume lowering effect may be attributed to insulin-induced increases in muscle capillary blood flow. Insulin concomitantly activates sympathetic nerves which also results in increased muscle blood flow. It has been reported that intravenous infusion of adrenaline causes an increase in venous Hct and a reduction in plasma volume [16]. On these bases, reduced plasma volume caused by the non-adrenergic and adrenergic effects of insulin may be one of reasons for the elevated Hct in subjects with insulin resistance. Although there is no conclusive answer about this issue, several previous studies also reported associations between adiposity and hemorheological alterations including blood viscosity [25-29], erythrocyte aggregation, deformability [25, 27], and

rigidity [29]. Proliferation of erythroid progenitor cells by insulin [10, 30, 31] may be another reason.

The pathogenesis of brain ischemic damage, including white matter burden, has not been fully elucidated. Chronic hypoperfusion resulting from small vessel damage, such as vessel lumen restriction and wall thickening, is thought to be a key factor in the degeneration of brain parenchyma [32]. In the present study, we showed that reduced Hct levels were associated with the presence of PVH. Several studies have suggested that chronic anemia may be a risk factor for incident stroke in patients with chronic kidney disease [33], for all-cause mortality in diabetic patients [34], and for white matter burden in hypertensive persons [35]. In addition to its role in subjects already at high cardiovascular risk, anemia has been suggested to be an independent risk factor for cardiovascular outcomes in the general population [36]. Several potential reasons explain the association between anemia and cardiovascular diseases. Firstly, chronic anemia (less than 10 g/dL) may lead to left ventricular hypertrophy [37] as an adaption to an anemia-related long-term increase in blood flow volume and pressure load. Secondly, anemia may cause myocardial ischemia [38]. However, it is uncertain whether low Hct levels in a general population could lead to such morphological changes or to ischemia in the cardiovascular system. In this regard, our present results showed that MCV and MCH levels were not associated with PVH. This result indicates that increased body fluid volume and consequent blood congestion might also plausibly explain the relationship between Hct level and PVH. Several findings to support this possibility have been reported. Chung et al reported that age-related white matter changes were more severe in persons with severe jugular venous reflux [39], while a community-based study reported the association of white matter lesions with impaired kidney function [40]. Although these latter authors suggested that small vessel damage was one factor relating kidney function and white matter lesions, the effects of body fluid cannot be excluded.

In summary, we found that a higher Hct in subjects with insulin resistance was an

epiphenomenon of adiposity. In clinical settings, however, Hct may nevertheless represent subclinical brain damage independent of other established risk factors. Since association between increased Hct and blood flow depends on endothelial functions [41, 42], additional studies with a large number of samples are required to set a cut-off point of Hct level. Strengthening of these findings awaits independent studies with longitudinal data.

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FIGURE LEGENDS

Figure 1 Association of Hct quartile with insulin resistance and insulin sensitivity

Study subjects were divided into quartiles by sex and then combined to avoid potential sex differences. The revised version of homeostasis model assessment index for insulin resistance (HOMA2-IR) [18] was used as an index of insulin resistance. Insulin sensitivity index (ISI) was calculated using Gutt's equation [19]. The number of subjects in each group is represented in the column. Statistical significance was assessed by analysis of variance.

Figure 2 Frequency differences of asymptomatic cerebrovascular disease by hematocrit and WBC quartile (AAC subjects)

Statistical significance was assessed by the chi-squared test. Subjects with periventricular hyperintensity (PVH) grade II or higher were considered as having PVH.

Table 1 Clinical characteristics of study subjects

	AAC (952)	THS (1,026)
Age (years)	66±9	60±11
BMI (kg/m^2)	23.1±3.0	23.3±3.0
Visceral fat area (cm^2)	103±60	-
Systolic BP (mmHg)	135±19	129±20
Diastolic BP (mmHg)	78±11	77±11
Glucose (mg/dl)	103±16	93±10
Insulin (μU/ml)	5.6±3.4	5.4±3.1
HOMA2-IR	0.75±0.45	0.71±0.41
ISI	-	1.96±0.69
HDL cholesterol (mg/dl)	68±18	60±14
ALT (IU/l)	22±11	21±10
Total protein (g/dl)	7.4±0.4	-
B-type natriuretic peptide (pg/ml)	31.7±30.9	-
Hematocrit (%)	42.5±3.5	39.8±3.8
Red blood cell count (x10^4/μl)	4.6±0.4	
MCV (fl)	92.5±4.1	-
MCH (pg)	31.0±1.7	-
White blood cell count (x10^9/litter)	5.35±1.39	5.34±1.34
baPWV (cm/sec.)	1577±329	-
Carotid IMT (mm)	0.79±0.15	-
PVH (grade 1/2/3/4)	409/74/9/2	-
Lacunar infarction (%)	9.6	-

The revised version of homeostasis model assessment index for insulin resistance (HOMA2-IR) was calculated from fasting insulin (pmol/l) and glucose (mmol/L) using a HOMA Calculator (http://www.dtu.ox.ac.uk/homacalculator/index.php) [18]. Insulin sensitivity index (ISI) was calculated according to Gutt's equation [19]: [75,000 mg + (glucose$_0$ - glucose$_{120}$) × 0.19 × body weight /120 min] / [(glucose$_0$ + glucose$_{120}$) / 2] / log [(insulin$_0$ + insulin$_{120}$) / 2]. Brachial-to-ankle pulse wave velocity (baPWV) was measured using a cuff-oscillometric device [23]. Intima-media thickness (IMT) at the common carotid artery was measured by ultrasonography. Lacunar infarction and periventricular hyperintensity (PVH) were evaluated by brain MRI with a 3-tesla scanner. BMI: body mass index, BP: blood pressure, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin. Sex differences were assessed by analysis of variance.

Table 2 Multiple linear regression analysis for HOMA-IR and ISI

	AAC				THS			
	HOMA2-IR				HOMA2-IR		ISI	
	Model 1		Model 2					
	β	p	β	p	β	p	β	p
Age (years)	<-0.001	0.995	-0.003	0.928	-0.025	0.368	-0.174	**<0.001**
Sex (male)	0.074	**0.033**	0.119	**<0.001**	0.221	**<0.001**	-0.104	**0.004**
BMI (kg/m^2)	0.375	**<0.001**	0.212	**<0.001**	0.451	**<0.001**	-0.216	**<0.001**
Systolic BP (mmHg)	0.032	0.288	0.008	0.789	0.018	0.529	-0.162	**<0.001**
HDL cholesterol (mg/dl)	-0.148	**<0.001**	-0.106	**0.001**	-0.181	**<0.001**	0.118	**<0.001**
Visceral fat area (cm^2)			0.221	**<0.001**				
ALT (IU/l)			0.165	**<0.001**				
White blood cell count (x10^9/litter)	0.114	**<0.001**	0.103	**<0.001**	0.119	**<0.001**	-0.125	**<0.001**
Hematocrit (%)	0.095	**0.007**	0.039	0.264	0.177	**<0.001**	-0.121	**<0.001**

Visceral fat area was measured from a CT image taken at the level of the umbilicus.

Table 3 Differences in hemodynamic parameters and arterial properties by hematocrit quartile (AAC subjects)

	Quartile of hematocrit				p
	Q1 (229)	Q2 (235)	Q3 (250)	Q4 (238)	
Whole blood viscosity (cP)	3.82±0.29	4.16±0.25	4.40±0.25	4.75±0.32	<0.001
Wall shear stress at systole (dyne/cm^2)	16.9±5.3	18.7±5.8	21.0±6.3	22.1±6.7	<0.001
Wall shear stress at end-diastole (dyne/cm^2)	5.1±2.0	5.7±2.0	6.3±2.1	6.6±2.4	<0.001
baPWV (cm/sec)	1580±348	1562±317	1555±302	1613±347	0.210
Carotid IMT (mm)	0.80±0.16	0.80±0.15	0.78±0.15	0.80±0.15	0.306

Whole blood viscosity at 208 seconds^{-1} was estimated by the following formula [21]: 0.12 x hematocrit (%) + 0.17 x plasma total protein (g/dl) – 2.07. Wall shear stress was calculated according to the Poisuille's law [22]: (4 x whole blood viscosity (poise) x blood velocity (cm/sec) / carotid diameter (mm). Differences by hematocrit quartile were assessed by analysis of variance.
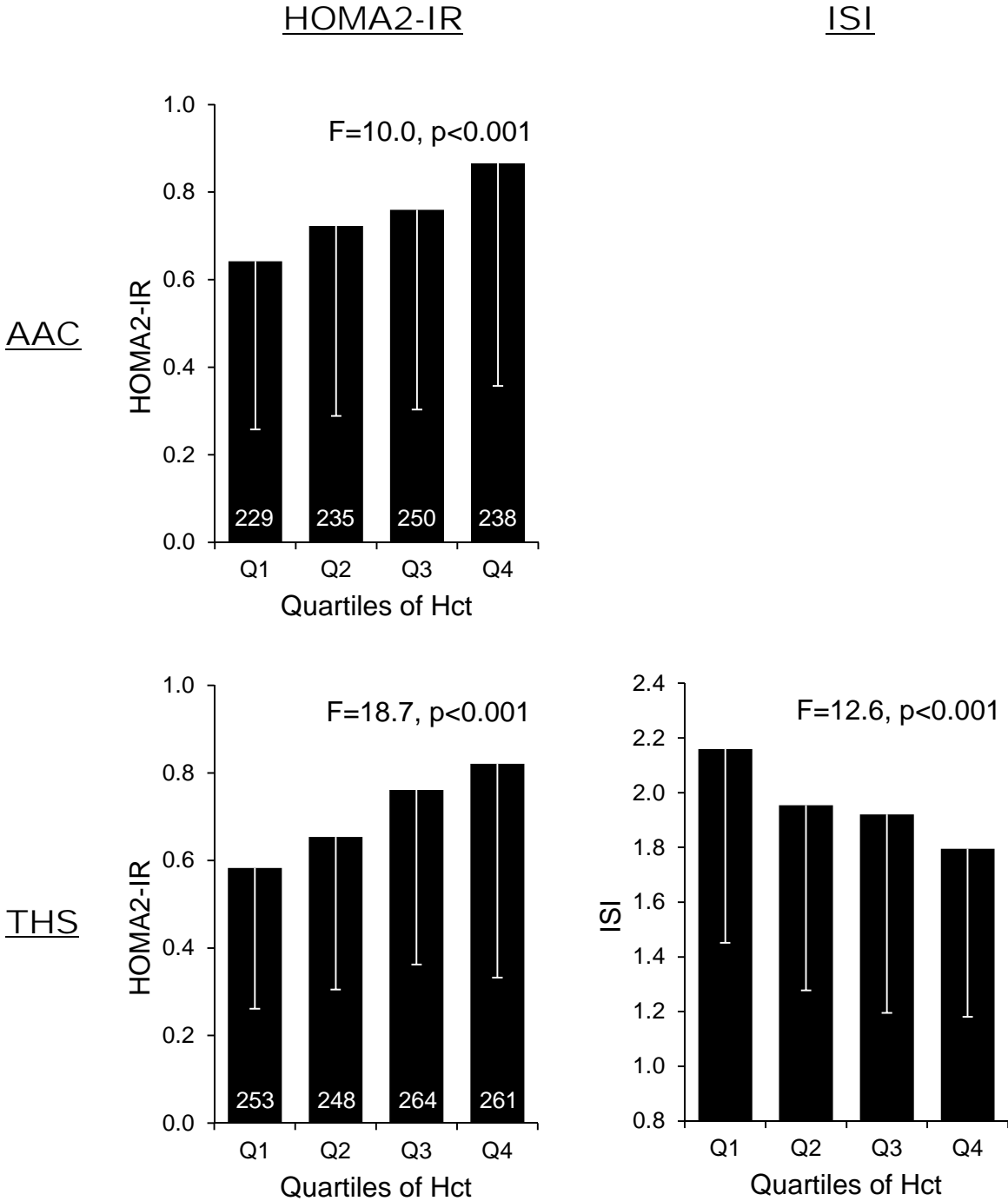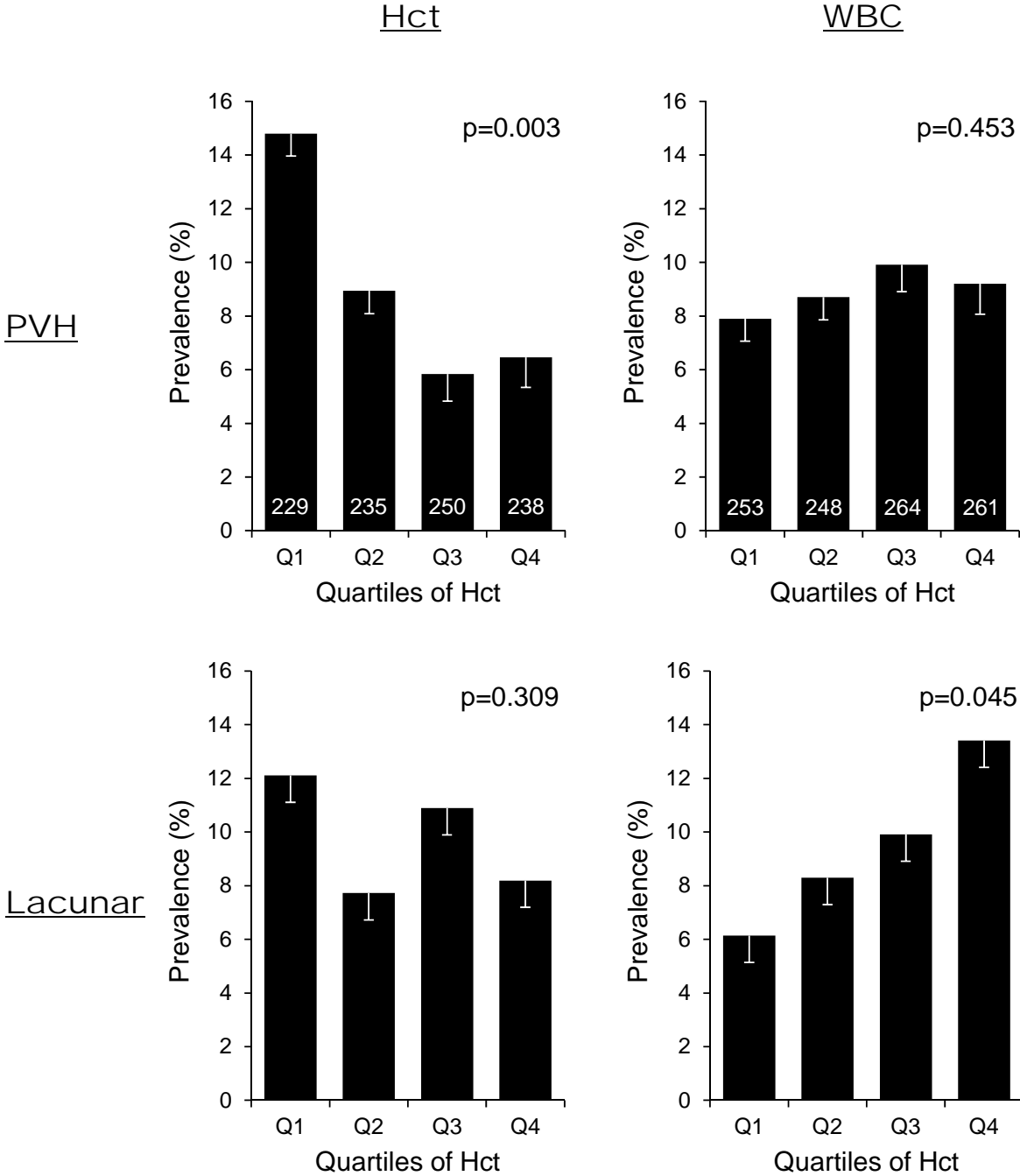
FIGURE 1

Figure 1. HOMA2-IR and ISI across quartiles of Hct in AAC and THS cohorts.

FIGURE 2

Hct

WBC

PVH

Lacunar

ONLINE SUPPLEMENT

Association of hematological parameters with insulin resistance, insulin sensitivity, and asymptomatic cerebrovascular disease: the J-SHIP Toon Health Study

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Supplemental Table 1　Clinical characteristics of AAC subjects

	Total (952)	Male (367)	Female (585)	p
Age (years)	66±9	66±10	65±9	0.074
BMI (kg/m^2)	23.1±3.0	23.7±2.8	22.8±3.0	<0.001
Visceral fat area (cm^2)	103±60	130±67	86±48	<0.001
Systolic BP (mmHg)	135±19	138±19	134±19	0.002
Diastolic BP (mmHg)	78±11	80±11	76±11	<0.001
Glucose (mg/dl)	103±16	107±18	100±14	<0.001
Insulin (μU/ml)	5.6±3.4	5.9±3.7	5.3±3.1	0.005
HOMA2-IR	0.75±0.45	0.81±0.50	0.71±0.42	0.002
HDL cholesterol (mg/dl)	68±18	61±17	72±18	<0.001
ALT (IU/l)	22±11	25±13	20±9	<0.001
Total protein (g/dl)	7.4±0.4	7.4±0.4	7.4±0.4	0.378
B-type natriuretic peptide (pg/ml)	31.7±30.9	29.6±30.3	33.1±31.2	0.088
Hematocrit (%)	42.5±3.5	44.9±3.2	41.0±2.7	<0.001
Red blood cell count (x10^4/μl)	4.6±0.4	4.8±0.4	4.5±0.3	<0.001
MCV (fl)	92.5±4.1	93.4±3.9	91.9±4.1	<0.001
MCH (pg)	31.0±1.7	31.6±1.7	30.6±1.6	<0.001
White blood cell count (x10^9/litter)	5.35±1.39	5.61±1.45	5.19±1.33	<0.001
baPWV (cm/sec.)	1577±329	1638±325	1540±325	<0.001
Carotid IMT (mm)	0.79±0.15	0.82±0.16	0.78±0.15	<0.001
PVH (grade 1/2/3/4)	409/74/9/2	158/31/4/0	251/43/5/2	0.773
Lacunar infarction (%)	9.6	9.5	9.6	0.985

The revised version of homeostasis model assessment index for insulin resistance (HOMA2-IR) was calculated from fasting insulin (pmol/l) and glucose (mmol/L) using a HOMA Calculator (http://www.dtu.ox.ac.uk/homacalculator/index.php) [18]. Brachial-to-ankle pulse wave velocity (baPWV) was measured using a cuff-oscillometric device [23]. Intima-media thickness (IMT) at the common carotid artery was measured by ultrasonography. Lacunar infarction and periventricular hyperintensity (PVH) were evaluated by brain MRI with a 3-tesla scanner. BMI: body mass index, BP: blood pressure, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin. Sex differences were assessed by analysis of variance.

Supplemental Table 2   Clinical characteristic of THS subjects

	Total (1,026)	Male (331)	Female (695)	p
Age (years)	60±11	61±12	59±12	0.006
BMI (kg/m^2)	23.3±3.0	23.9±2.3	23.0±3.2	<0.001
Systolic BP (mmHg)	129±20	131±18	128±20	0.058
Diastolic BP (mmHg)	77±11	80±11	75±11	<0.001
Glucose (mg/dl)	93±10	96±10	91±9	<0.001
Insulin (μU/ml)	5.4±3.1	2.5±2.3	2.8±3.1	0.162
HOMA2-IR	0.71±0.41	0.72±0.42	0.70±0.40	0.360
ISI	1.96±0.69	1.87±0.64	2.00±0.71	0.007
HDL cholesterol (mg/dl)	60±14	54±13	63±14	<0.001
ALT (IU/l)	21±10	24±11	19±10	<0.001
Hematocrit (%)	39.8±3.8	43.1±3.1	38.2±3.0	<0.001
White blood cell count (x10^9/litter)	5.34±1.34	5.72±1.42	5.16±1.27	<0.001

The revised version of homeostasis model assessment index for insulin resistance (HOMA2-IR) was calculated from fasting insulin (pmol/l) and glucose (mmol/L) using a HOMA Calculator (http://www.dtu.ox.ac.uk/homacalculator/index.php) [18]. Insulin sensitivity index (ISI) was calculated according to Gutt's equation [19]: [75,000 mg + (glucose$_0$ - glucose$_{120}$) × 0.19 × body weight /120 min] / [(glucose$_0$ + glucose$_{120}$) / 2] / log [(insulin$_0$ + insulin$_{120}$) / 2]. Sex differences were assessed by analysis of variance.

Supplemental Table 3   Hemodynamic characteristic of AAC subjects

|	Total (952)	Male (367)	Female (585)	p
Whole blood viscosity (cP)	4.29±0.44	4.58±0.41	4.11±0.35	<0.001
Carotid diameter (mm)				
Systole	6.6±0.8	7.0±0.9	6.3±0.7	<0.001
End-diastole	6.1±0.8	6.5±0.9	5.9±0.7	<0.001
Blood velocity (cm/sec.)				
Systole	73.5±16.6	74.0±17.7	73.2±16.0	0.486
End-diastole	20.4±5.8	18.2±5.5	21.8±5.6	<0.001
Wall shear stress (dyne/cm^2)				
Systole	19.7±6.4	20.1±6.9	19.5±6.0	0.177
End-diastole	5.9±2.2	5.4±2.2	6.3±2.1	<0.001

Whole blood viscosity at 208 seconds^{-1} was estimated by the following formula [21]: 0.12 x hematocrit (%) + 0.17 x plasma total protein (g/dl) – 2.07. Common carotid diameters were measured by ultrasonography. Carotid blood velocity was measured by Doppler ultrasonography. Wall shear stress was calculated according to Poisuille's law [22]: (4 x whole blood viscosity (poise) x blood velocity (cm/sec) / carotid diameter (mm). Sex differences were assessed by analysis of variance.

Supplemental Table 4   Differences in clinical parameters by hematocrit quartile (AAC subjects)

	Quartile of hematocrit				p
	Q1 (229)	Q2 (235)	Q3 (250)	Q4 (238)	
Age (years)	67±10	67±9	65±9	65±8	0.001
Visceral fat area (cm^2)	87±58	102±60	104±56	119±62	<0.001
BMI (kg/m^2)	22.5±2.8	23.0±2.9	23.1±3.0	23.8±2.9	<0.001
Systolic BP(mmHg)	133±19	134±19	136±19	139±21	0.013
Diastolic BP (mmHg)	74±11	77±11	78±10	81±11	<0.001
HDL cholesterol (mg/dl)	69±19	67±17	68±19	66±17	0.414
ALT (IU/l)	19±8	21±11	23±11	25±13	<0.001
Glucose (mg/dl)	101±15	100±12	103±14	107±21	<0.001
B-type natriuretic peptide (pg/ml)	40.4±32.9	29.9±25.1	30.1±32.2	26.9±31.3	<0.001

Group difference was assessed by analysis of variance.
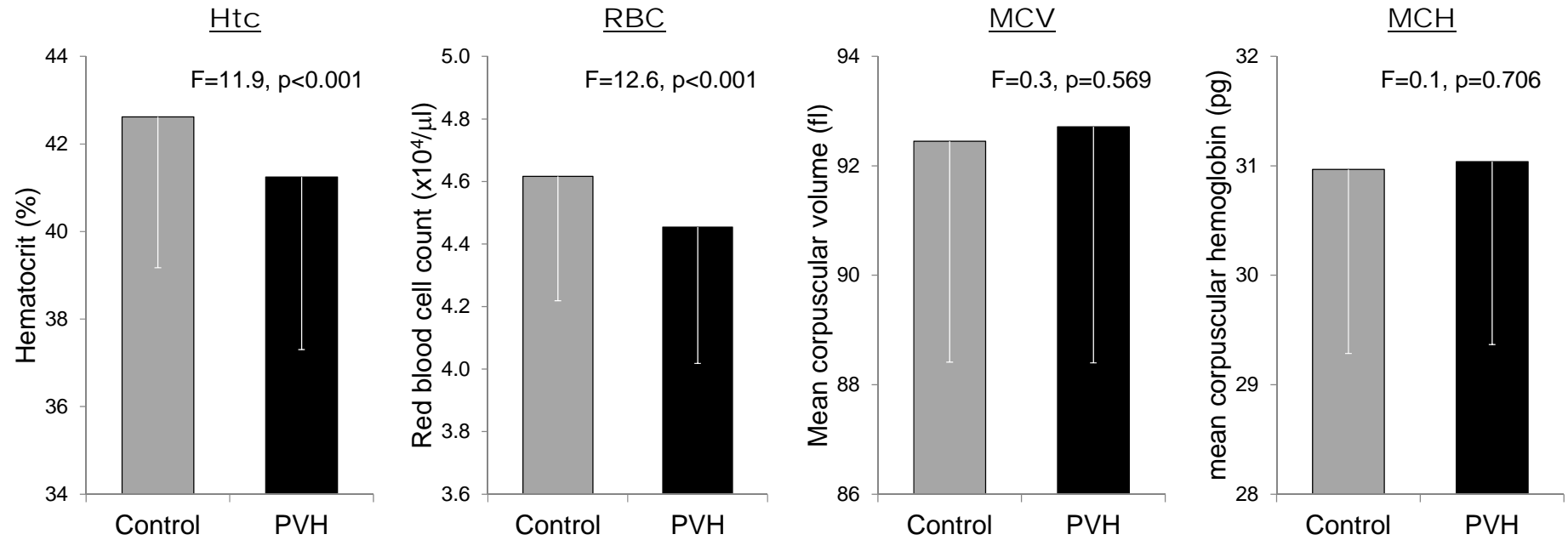
Supplemental figure 1 Association between PVH and erythrocyte index

Subjects with PVH grade 2 or higher was considered as having PVH. Statistical significance was assessed by analysis of variance. Hct: hematocrit, RBC: red blood cell count, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin.