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Hyperinsulinemia and Hemostatic Abnormalities Are Associated With Silent Lacunar Cerebral Infarcts in Elderly Hypertensive Subjects

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OBJECTIVES We sought to study the association of the silent cerebral infarct (SCI), a predisposing condition of stroke, with hyperinsulinemia and hemostatic abnormalities in older hypertensive subjects.

BACKGROUND Hypertension is a powerful risk factor for stroke. However, the role of other risk factors for stroke in hypertensive subjects remains incompletely understood.

METHODS We performed brain magnetic resonance imaging and measured cardiovascular risk factors, by administering the 75-g oral glucose tolerance test and measuring plasma insulin and hemostatic variables, in 123 asymptomatic hypertensive subjects (mean age 69 years).

RESULTS At least one SCI was detected in 80 subjects (65%), and multiple SCIs were found in 48 subjects (39%). The presence of SCIs was associated with older age, higher levels of 24-h systolic blood pressure, 2-h insulin, thrombin-generation markers (prothrombin fragment 1+2 and thrombin-antithrombin complexes), plasminogen activator inhibitor-1 (PAI-1), D-dimer and von Willebrand factor (vWF), but not with plasmin-alpha$_2$-plasmin complex (PIC) levels. The 2-h insulin area under the curve (AUC) was positively correlated with PAI-1 and vWF levels ($p < 0.01$), and the PAI-1 level was negatively correlated with the PIC level ($p < 0.02$). Multiple logistic regression analysis revealed that age and the 2-h insulin AUC were significantly associated with SCIs, particularly those located in the subcortical white matter, and hemostatic abnormalities were significantly associated with the presence of multiple SCIs, particularly those located in the basal ganglia.

CONCLUSIONS In older asymptomatic hypertensive subjects, hyperinsulinemia appears to be associated with lacunar-type SCIs, particularly those located in the subcortical white matter, and hemostatic abnormalities show an association with the presence of multiple SCIs, particularly those located in the basal ganglia. (J Am Coll Cardiol 2001;37:871–7) © 2001 by the American College of Cardiology

Hypertension is a powerful risk factor for stroke. However, the role of other risk factors for stroke in hypertensive subjects remains incompletely understood. In hypertensive subjects, clustering abnormalities of lipid and glucose metabolism and impaired fibrinolysis have been reported as a part of insulin resistance syndrome (1,2). Insulin resistance syndrome and hemostatic abnormalities (imbalance between coagulation and fibrinolysis) have been proposed as new risk factors for cardiovascular events (1). Plasma insulin profiles are indicators of insulin resistance syndrome, and hyperinsulinemia is related to impaired fibrinolysis (3,4). Fibrinolytic activity is determined by the balance between tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1), both of which are synthesized and secreted from endothelial cells (ECs). Endothelial cell damage also affects the imbalance in fibrinolysis.

Unlike coronary artery disease, the effects of insulin resistance syndrome, hemostatic abnormalities and EC damage on stroke have not yet been thoroughly investigated. The silent cerebral infarct (SCI) is now thought to be a predisposing condition for clinically overt stroke (5). One prospective study demonstrated that SCI was a strong predictor of subsequent clinically overt stroke, with an odds ratio of ~10 (6). As hemostatic factors and EC factors are complicated by changes that occur due to brain tissue damage after clinical stroke events (7,8), we used SCI as a surrogate model for ischemic stroke, because such minor brain damage is less likely to influence hemostatic and EC factors. We have recently found increased thrombin generation in SCIs of elderly subjects with risk factors (9). However, there are no reports on the relationships between hyperinsulinemia, hemostatic abnormalities and SCI in asymptomatic hypertensive subjects.

To study these relationships, we performed the 75-g oral glucose tolerance test (OGTT) and brain magnetic resonance imaging (MRI) and measured insulin and

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Abbreviations and Acronyms

AUC = area under the curve
EC = endothelial cell
ELISA = enzyme-linked immunosorbent assay
F1+2 = prothrombin fragment 1+2
MRI = magnetic resonance imaging
OGTT = 75-g oral glucose tolerance test
PAI-1 = plasminogen activator inhibitor-1
PIC = plasmin-alpha$_2$-plasmin inhibitor complex
SCI = silent cerebral infarct
TAT = thrombin-antithrombin complex
t-PA = tissue-type plasminogen activator
vWF = von Willebrand factor

hemostatic variables in asymptomatic elderly hypertensive patients.

METHODS

Patients

We studied 123 asymptomatic hypertensive subjects (age ≥55 years, range 55 to 88) who were recruited from outpatient clinics. Hypertension was diagnosed when clinical systolic blood pressure was consistently ≥140 mm Hg and/or clinical diastolic blood pressure was ≥90 mm Hg at three or more visits. Clinical blood pressure was measured in the sitting position after the subject had rested for at least 5 min. The subjects were referred to the clinics because of high blood pressure, were identified from population-based screening for cardiovascular risk factors or were treated for mild hypertension (they had agreed to stop antihypertensive medications for ambulatory blood pressure monitoring and blood testing, n = 27). None of the patients had received antihypertensive or lipid-lowering medications for at least two weeks before this study. Of the 123 subjects studied, 77 (63%) had a history of antihypertensive treatment. Excluded from this study were patients with renal failure (serum creatinine > 106 μmol/liter), hepatic damage or obvious present and/or previous coronary artery disease, stroke, congestive heart failure, atrial fibrillation or malignancy. Patients who showed signs of overt diabetes mellitus (fasting glucose >7.8 mmol/liter and/or hemoglobin A_{1c} >6.4%) were also excluded. All of the subjects were ambulant and had a normal appetite. The results of the physical examination and laboratory studies (blood and urine tests, chest X-ray and rest electrocardiography) were normal. No cervical bruit was audible in the subjects. Smokers and nonsmokers were defined by their current smoking status. Body mass index was calculated as weight (kg)/height (m^2). Written, informed consent was obtained from all subjects. This study was approved by the Research Ethics Committee, Department of Cardiology, Jichi Medical School, Japan.

Brain MRI and Patient Classification

Brain MRI was carried out with a 1.5-tesla MRI unit (Toshiba MRT200FXII, Tokyo, Japan). The brain was imaged in the axial plane at 8-mm slice thickness. An SCI was defined exclusively as a low signal intensity area (≥3 mm in size), depicted on T1-weighted images, that was also visible as a hyperintense lesion on T2-weighted images (Fig. 1). The number and location of SCIs in the territory of the carotid artery system were assessed in each subject. All SCIs detected were lacunar infarcts <15 mm. We classified the asymptomatic hypertensive subjects studied into the three following subgroups, based on the number of SCIs: 1) noninfarct group with no SCI; 2) few infarct group with one or two SCIs per person; and 3) multiple infarct group with three or more SCIs per person (10). The location of SCIs was divided into the following two subtypes: 1) subcortical white matter infarct; and 2) basal ganglia infarct (head of caudate, putamen, pallidum and thalamus). All of the MRIs were interpreted in a blinded manner, and classification of the patients was done before the blood testing.

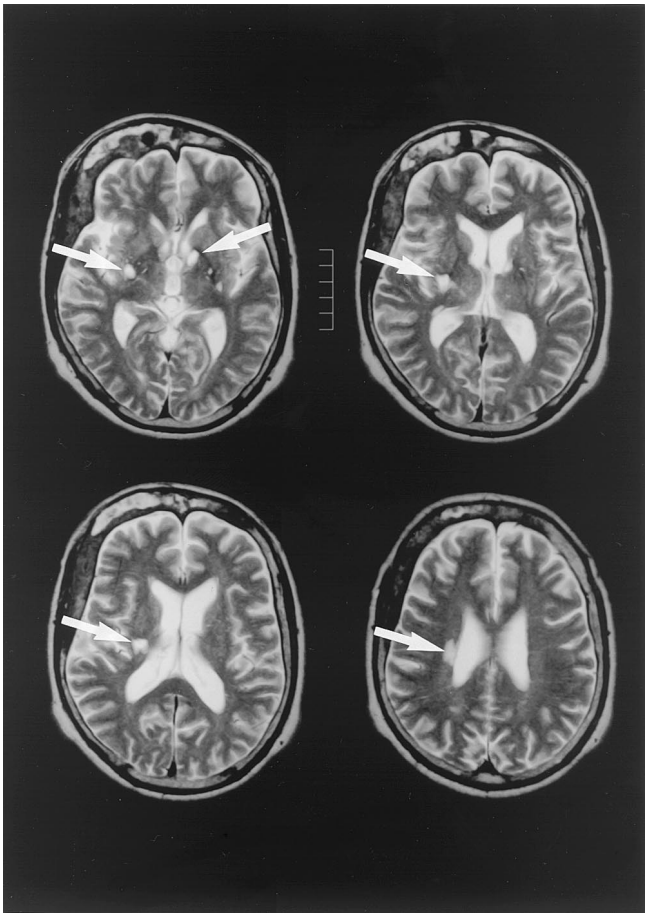

Figure 1. Brain MRI findings (T2-weighted images) of silent multiple lacunar infarcts. The **top images** show lacunar infarcts **(arrows)** in the basal ganglia, and the **lower images** show those infarcts **(arrows)** in the deep white matter. Cerebral infarcts were defined exclusively as a low signal intensity area (3 to 15 mm in size), depicted on T1-weighted images, that was also visible as a hyperintense lesion on T2-weighted images. MRI = magnetic resonance imaging.

Ambulatory Blood Pressure Monitoring and Electrocardiography

Noninvasive ambulatory blood pressure monitoring was carried out on a weekday using an automatic system with gas-powered cuff inflation (ABPM-630, Nippon Colin Co., Komaki, Japan), which recorded blood pressure and heart rate every 30 min for 24 h (11). In five patients, the initial blood pressure data were rejected because of artifacts in >10% of the total measurements; however, the examination was repeated, and the second set of blood pressure data was included in the analysis. Left ventricular hypertrophy, as shown by electrocardiography, was defined as abnormally high QRS voltages (R wave in lead V_5 plus S wave in lead V_1 ≥3.5 mV) associated with either flat T waves (<10% of R wave) or ST segment depression (>0.1 mV) and biphasic T waves.

Sample Collection

Blood samples were obtained between 9 AM and 10 AM after an overnight fast on the same day that the OGTT was performed. Specimens for the assay of coagulation variables were collected by the two-syringe method into disposable siliconized vacuum glass tubes containing a 1/10 volume of 3.8% trisodium citrate. The samples were centrifuged at 3,000 *g* for 15 min at room temperature. After separation, the serum samples for lipids were stored at 4°C in refrigerated containers if the analysis was not performed within the next few days. The sample for coagulation and insulin assays was subsequently separated and stored in several plastic tubes at −80°C until laboratory tests were performed.

Oral Glucose Tolerance-Test

For the OGTT, a 75-g glucose load was administered after a 12-h overnight fast. Blood was drawn immediately before ingestion and 30, 60 and 120 min after the glucose load. The area under the curve (AUC) of the plasma glucose–time plot (2-h glucose AUC) and that of the insulin–time plot (2-h insulin AUC) were calculated.

Assay Procedure

Plasma fibrinogen levels were determined using a one-stage clotting assay kit (Data-Fi, Dade, Florida) (12). The plasma levels of PAI-1, t-PA–PAI-1 complex, plasmin-alpha$_2$-plasmin inhibitor complex (PIC), thrombin–antithrombin complex (TAT), prothrombin fragment 1+2 (F1+2) and D-dimer were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits for PAI-1 (Biopool, Sweden), t-PA–PAI-1 complex and PIC (Teijin Co. Inc., Tokyo, Japan), F1+2 and TAT (Behringwerke AG, Marburg, Germany) and D-dimer (Diagnostica Stago, Asnières, France). The plasma level of von Willebrand factor (vWF) was measured by using the recently developed ELISA kit (Shield Diagnostics Ltd., Dundee, United Kingdom) using monoclonal antibody against the functional epitope of vWF (13), and the value for commercially available pooled plasma (CTS Standard Plasma, Behringwerke AG, Germany) was taken as 100%. Serum insulin was determined by a radioimmunoassay kit (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

Statistical Analysis

All analyses were conducted with SPSS/Windows, version 8.0J (SPSS Inc.). The mean values and proportions were calculated for background variables. Geometric mean values ± SD were calculated for triglycerides, insulin, glucose, vWF, PAI-1, t-PA–PAI-1 complex, PIC, F1+2, TAT, D-dimer and fibrinogen levels owing to skewed distribution of the variables. After one-way analysis of variance, the Scheffé *F* test was used for comparisons of continuous variables among the groups. The unpaired *t* test was used for comparisons of continuous variables between the two groups. The chi-square or Fisher exact test was used for calculating proportions. Pearson correlation coefficients were used to measure correlations between continuous variables. Adjusted odds ratios with 95% confidence intervals for no or low SCI (0 = those with no infarct; 1 = those with one or more infarcts) and for multiple SCIs (0 = those with <3 infarcts; 1 = those with ≥3 infarcts) were calculated by using multiple logistic regression analysis with selected independent variables. To study the relationship between the location of the SCI and cardiovascular risk factors, we conducted multiple logistic analysis with white matter infarction (0 = those with no infarct and those with only a basal ganglia infarct; 1 = those with a white matter infarct both with and without a basal ganglia infarct) or basal ganglia infarction (0 = those with no infarct and those only with a white matter infarct; 1 = those with a basal ganglia infarct both with and without a white matter infarct) as the dependent variable. Two-tailed tests were used, and p values <0.05 were considered significant.

RESULTS

Clinical Variables and SCI of Study Subjects

The mean ± SD clinical blood pressure levels of the total study group were 169 ± 22 mm Hg systolic and 97 ± 15 mm Hg diastolic, and the mean 24-h blood pressure levels were 144 ± 15 mm Hg systolic and 81 ± 11 mm Hg diastolic. Of the 123 study subjects 93% had hypertension according to the criteria of the American Society of Hypertension (14). The prevalence of total SCI was 65% (n = 80), and that of multiple SCIs was 39% in the total study group.

Table 1 shows the characteristics of the 123 subjects separated into three groups according to the number of SCIs. The age was older and the 24-h systolic blood pressure was significantly higher in the total infarct group and in the multiple infarct group than in the noninfarct group, although there were no significant differences in clinical blood pressure levels between the three groups. The prevalence of smokers and left ventricular hypertrophy tended to be higher in the total infarct and multiple infarct

Table 1. Characteristics of Hypertensive Subjects With and Without a Silent Cerebral Infarct

Variables	Noninfarct Group (n = 43)	Total Infarct Group (n = 80)	Few Infarct Group (n = 32)	Multiple Infarct Group (n = 48)
Age (yrs)	66 ± 6.0	70 ± 7.9*	69 ± 7.6	71 ± 8.0*
Male	28%	31%	25%	35%
Body mass index (kg/m^2)	23.9 ± 3.6	24.4 ± 3.7	24.5 ± 4.3	24.3 ± 3.3
Clinical SBP (mm Hg)	167 ± 20	170 ± 22	169 ± 21	172 ± 23
Clinical DBP (mm Hg)	97 ± 13	98 ± 15	97 ± 17	98 ± 14
24-h SBP (mm Hg)	140 ± 13	146 ± 16†	142 ± 17	149 ± 14†
24-h DBP (mm Hg)	80 ± 10	81 ± 12	81 ± 12	82 ± 12
Smokers	30%	34%	28%	37%
ECG-LVH	14%	24%	22%	25%
Hematocrit	0.40 ± 0.03	0.40 ± 0.04	0.40 ± 0.04	0.40 ± 0.03
Total cholesterol (mmol/liter)	5.5 ± 0.6	5.2 ± 0.9	5.1 ± 0.8	5.3 ± 1.0
HDL cholesterol (mmol/liter)	1.3 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	1.2 ± 0.3
Triglycerides (mmol/liter)	1.5 (0.9–2.6)	1.5 (0.9–2.5)	1.4 (0.9–2.2)	1.6 (0.9–2.7)

*$p < 0.01$ and †$p < 0.05$ versus noninfarct group. The difference between the noninfarct group and the total infarct group was tested by using the unpaired *t* test. The differences between the noninfarct, few infarct and multiple infarct groups were tested by using the Scheffé *F* test after analysis of variance. The total infarct group was the combined group of the few infarct and multiple infarct groups; subjects with no cerebral infarct on brain magnetic resonance imaging were classified as the noninfarct group; subjects with one or two infarcts comprised the few infarct group; and subjects with three or more infarcts comprised the multiple infarct group. Data are presented as the mean value ± SD or the prevalence of other risk factors, except for triglycerides, expressed as the geometric mean value (SD range).

DBP = diastolic blood pressure; ECG-LVH = left ventricular hypertrophy detected by electrocardiography; HDL = high density lipoprotein; SBP = systolic blood pressure.

groups, although these did not reach statistical significance. The lipid profile was not different between the three groups.

Insulin and Glucose Profiles of OGTT

The 2-h insulin levels were significantly higher in the few infarct and multiple infarct groups than in the noninfarct group, and the 2-h insulin AUC was also higher in the multiple infarct group than in the noninfarct group or the few infarct group (Table 2). There were no significant differences in these insulin profiles between the noninfarct group and few infarct group. The glucose profiles were not different between the three groups.

Hemostatic Profiles

The levels of vWF, PAI-1, F1+2, TAT and D-dimer were significantly higher in the total infarct and multiple infarct groups than in the noninfarct group (Table 2). The vWF and PAI-1 levels were also higher in the multiple infarct group than in the few infarct group.

Association Between Insulin Profiles and Hemostatic Profiles

The 2-h insulin AUC was negatively correlated with high density lipoprotein cholesterol ($r = -0.25$, $p < 0.01$) and positively correlated with triglycerides ($r = 0.24$, $p < 0.01$), vWF ($r = 0.25$, $p < 0.01$), PAI-1 ($r = 0.28$, $p < 0.01$) and t-PA–PAI-1 complex ($r = 0.25$, $p < 0.01$). The fasting insulin level and 2-h insulin level had similar trends in their relationships with these factors. The 2-h insulin AUC tended to be negatively correlated with the PIC level ($r = -0.15$, $p < 0.07$). The plasma PAI-1 level was negatively correlated with the PIC level ($r = -0.22$, $p < 0.02$).

Table 2. Insulin Profiles and Hemostatic Factors in Hypertensive Subjects

Variables	Noninfarct Group (n = 43)	Total Infarct Group (n = 80)	Few Infarct Group (n = 32)	Multiple Infarct Group (n = 48)
Fasting plasma glucose (mmol/liter)	5.3 (4.1–6.1)	5.5 (4.8–6.2)	5.6 (4.9–6.3)	5.4 (4.8–6.1)
2-h plasma glucose (mmol/liter)	6.4 (5.0–9.8)	6.8 (4.8–9.6)	6.9 (4.9–9.8)	6.7 (4.7–9.5)
Fasting insulin (mmol/liter)	35 (25–50)	39 (28–59)	41 (28–60)	38 (25–58)
2-h plasma insulin (mmol/liter)	132 (66–252)	184 (90–376)†	174 (84–360)	192 (96–390)‡
2-h glucose AUC (pmol/liter per min)	7.4 (5.9–9.2)	7.9 (6.2–10)	8.1 (6.2–11)	7.8 (6.2–9.9)
2-h insulin AUC (pmol/liter per min)	159 (108–237)	184 (105–322)	153 (84–276)	207 (126–345)‡§
vWF (%)	138 (108–177)	171 (134–218)*	157 (122–203)	180 (144–225)*§
PAI-1 (μg/liter)	39 (21–72)	54 (28–104)†	42 (24–73)	64 (33–124)*§
t-PA–PAI-1 (μg/liter)	11 (7.1–16)	13 (7.7–21)	13 (8.2–21)	13 (7.2–21)
PIC (mg/liter)	1.2 (0.8–1.8)	1.4 (1.0–2.0)	1.4 (1.0–2.1)	1.4 (1.0–2.0)
F1+2 (nmol/liter)	1.3 (0.8–2.2)	1.6 (1.1–2.5)‡	1.5 (1.0–2.1)	1.7 (1.1–2.7)‡
TAT (μg/liter)	1.7 (1.1–2.9)	2.3 (1.4–4.0)†	2.0 (1.2–3.5)	2.6 (1.5–4.4)†
D-dimer (μg/liter)	184 (111–305)	291 (140–606)*	275 (158–479)‡	302 (131–695)†
Fibrinogen (g/liter)	2.66 (2.01–3.44)	2.88 (2.34–3.54)	2.82 (2.31–3.44)	2.91 (2.36–3.61)

*$p < 0.01$; †$p < 0.01$. ‡$p < 0.05$ versus noninfarct group; §$p < 0.05$ versus few infarct group. The difference between the noninfarct group and the total infarct group was tested by using the unpaired *t* test. The differences between the noninfarct, few infarct and multiple infarct groups were tested by using the Scheffé *F* test after analysis of variance. For the definitions of the total infarct, noninfarct, few infarct and multiple infarct groups, see Table 1. Data are expressed as the geometric mean value (SD range).

AUC = area under the curve (during the oral glucose tolerance test); F1+2 = prothrombin fragment 1+2; PAI-1 = plasmin activator inhibitor-1; PIC = plasmin-alpha$_2$-plasmin inhibitor complex; t-PA–PAI-1 = tissue-type plasminogen activator–PAI-1 complex; TAT = thrombin–antithrombin complex; vWF = von Willebrand factor.

Table 3. Multiple Logistic Regression Analysis With All Silent Cerebral Infarcts or Silent Multiple Cerebral Infarcts as the Dependent Variable in Hypertensive Subjects

Variables	All Cerebral Infarcts (n = 80)	Multiple Cerebral Infarcts (n = 48)
Age (10-yr groups)	2.9 (1.4–6.1)†	4.1 (1.5–9.0)†
Male	2.2 (0.80–6.6)	3.2 (0.87–12)
24-h SBP (10 mm Hg)	1.3 (0.98–1.8)	1.6 (1.2–2.3)†
2-h insulin AUC	8.1 (1.3–50)‡	3.1 (0.66–14)
vWF	1.5 (0.32–7.0)	5.7 (1.2–27)‡
PAI-1	2.7 (0.80–9.5)	13 (3.5–51)*
F1+2	1.3 (0.43–4.1)	5.6 (1.6–20)†
TAT	1.5 (0.52–4.5)	0.89 (0.28–2.9)
D-dimer	7.1 (0.82–61)	4.0 (0.97–16)

*$p < 0.001$; †$p < 0.01$; ‡$p < 0.05$. The adjusted odds ratios (95% confidence intervals) for silent cerebral infarcts (0 = subjects with no infarct; 1 = subjects with one or more infarcts) or for silent multiple cerebral infarcts (0 = subjects with less than three infarcts; 1 = subjects with three or more infarcts) were calculated by using multiple logistic regression analysis. The analyses of 2-h insulin area under the curve (AUC), von Willebrand factor (vWF), plasmin activator inhibitor-1 (PAI-1), prothrombin fragment 1+2 (F1+2), thrombin–antithrombin complex (TAT) and D-dimer were based on the following coding: 0 = less than the geometric mean value ± 1 SD; 1 = greater than or equal to the geometric mean value ± 1 SD. The geometric mean values ± 1 SD were 291 pmol/liter per min for 2-h insulin AUC, 207% for vWF, 93 μg/liter for PAI-1, 2.4 nmol/liter for F1+2; 3.6 μg/liter for TAT and 497 μg/liter for D-dimer.

SBP = systolic blood pressure.

Multiple SCIs Versus Any SCIs

Multiple logistic regression analysis was conducted using the factors that were significantly higher in the total infarct and multiple infarct groups than in the noninfarct group, as shown in Tables 1 and 2, as the independent variables (Tables 3 and 4). Only age and 2-h insulin AUC were significantly and independently associated with the end point of (any) SCI. However, for the end point of multiple SCIs, the list of variables showing a statistically significant independent association included age, 24-h systolic blood pressure, vWF, PAI-1 and F1+2, but not 2-h insulin AUC.

Table 4. Multiple Logistic Regression Analysis of Silent Subcortical Cerebral Infarcts or Silent Basal Ganglia Infarcts as the Dependent Variable in Hypertensive Subjects

Variables	White Matter Infarct (n = 57)	Basal Ganglia Infarct (n = 47)
Age (10-yr groups)	2.3 (1.2–4.2)*	1.1 (0.63–2.1)
Male	1.7 (0.67–4.5)	1.7 (0.64–4.4)
24-h SBP (10 mm Hg)	1.1 (0.83–1.4)	1.2 (0.63–2.1)
2-h insulin AUC	4.2 (1.1–16)†	0.98 (0.27–3.5)
vWF	1.1 (0.33–3.9)	2.4 (0.68–8.4)
PAI-1	2.2 (0.82–5.9)	2.8 (1.0–7.6)†
F1+2	2.3 (0.85–6.3)	0.46 (0.15–1.4)
TAT	1.2 (0.47–2.9)	1.8 (0.71–4.5)
D-dimer	1.2 (0.34–3.9)	4.0 (1.1–14)†

*$p < 0.01$. †$p < 0.05$. The adjusted odds ratios (95% confidence intervals) for a subcortical white matter infarct (0 = subjects with no infarct and those with only a basal ganglia infarct; 1 = those with a white matter infarct both with and without a basal ganglia infarct) or for a basal ganglia infarct (0 = subjects with no infarct and those with only a white matter infarct; 1 = subjects with a basal ganglia infarct both with and without a white matter infarct) were calculated by using multiple logistic regression analysis with selected independent variables. The analyses of 2-h insulin area under the curve (AUC), von Willebrand factor (vWF), plasmin activator inhibitor-1 (PAI-1), prothrombin fragment 1+2 (F1+2), thrombin–antithrombin complex (TAT) and D-dimer were based on the following coding: 0 = less than the geometric mean value ± 1 SD; 1 = greater than or equal to the geometric mean value ± 1 SD. The geometric mean values ± 1 SD were 291 pmol/liter per min for 2-h insulin AUC, 207% for vWF, 93 μg/liter for PAI-1, 2.4 nmol/liter for F1+2, 3.6 μg/liter for TAT and 497 μg/liter for D-dimer. Basal ganglia infarcts include infarcts of the caudate, putamen, globus pallidum and thalamus.

SBP = systolic blood pressure.

Location of SCIs

Of the 80 subjects with one or more SCIs, 35 (44%) had infarcts only in the white matter, 25 (31%) had them only in the basal ganglia and 22 (28%) had infarcts in both. In the separate analysis of subtypes according to location (Table 4), we found that a white matter infarct was independently associated with aging and 2-h insulin AUC, and a basal ganglia infarct was independently associated with PAI-1 and D-dimer levels. When smoking and lipid profiles (total cholesterol, high density lipoprotein cholesterol and triglycerides) were selected as independent variables for each model, these variables were not related to either white matter or basal ganglia infarcts (data not shown).

DISCUSSION

Several novel findings emerged from our study of Japanese elderly hypertensive subjects. We found that hyperinsulinemia was associated with lacunar SCIs detected by brain MRI, particularly those located in the white matter. Furthermore, we found that hemostatic abnormalities were associated with multiple SCIs, particularly those located with the basal ganglia.

Prevalence of SCIs and Their Location

In this study, the prevalence of SCIs in the total study group was 65%. In a previous study, one or more SCIs were detected in 14 (41%) of 34 normotensive, healthy, elderly subjects recruited from a Japanese community (10). In the previous MRI studies of high risk elderly Japanese subjects, the prevalence of SCI was ~50% (10,15,16). In our present study, the prevalence of SCI (65%) was slightly higher than these previous results. This may be due to higher blood pressure levels in our group as compared with others. According to the criteria of the American Society of Hypertension, 93% of our study group had hypertension in which both clinical and ambulatory blood pressures were high. In a previous MRI study of 219 Japanese adults (mean age 63 years, 64% men) with no history of stroke or transient ischemic attack who visited a neurologic department, the investigators detected one or more SCIs in 88 subjects (40%), and the distribution was as follows: 50 (57%) had white matter infarcts, 6 (6.8%) had only basal ganglia infarcts and 32 (36%) had both (17). In our study, of the 80 subjects with one or more SCIs, 35 (44%) had infarcts only in the white matter, 25 (31%) had them only in the basal ganglia and 22 (28%) had SCIs in both. The basal ganglia infarcts were more common, and white matter infarcts were less common in our group, as compared with the previous study.

Association of Hyperinsulinemia with SCIs

In the present study, all SCIs were lacunar infarcts <15 mm in size, indicating that hyperinsulinemia may be related to small-vessel disease. In previous studies of insulin levels or underlying insulin resistance and symptomatic lacunar infarction, the results were inconsistent (18,19). One study found that fasting plasma insulin levels were higher in the lacunar stroke group than in the large-vessel disease group and the control group (18), whereas another study found that only the atherothrombotic stroke group had a higher insulin resistance (directly determined by the steady state plasma glucose method) and 2-h insulin AUC during OGTT, as compared with the lacunar stroke, cardioembolic stroke and control groups (19). The hemodynamic effect may also be a confounding factor that may explain the positive relationship between hyperinsulinemia and SCI. In one previous study, the intima-medial thickness of the carotid artery was positively associated with hyperinsulinemia (20). Advanced atherosclerosis and the related increase in stiffness of the large artery may cause enhanced fluctuation of cerebral blood flow in the marginal arteries.

Association Between Hyperinsulinemia and Hemostatic Abnormalities

Insulin profiles (fasting insulin, 2-h insulin and/or 2-h insulin AUC) were associated positively with plasma levels of PAI-1, other EC-derived factors (vWF and t-PA–PAI-1 complex) and triglycerides, and negatively with the high density lipoprotein cholesterol level. These factors may be confounding factors that may partly explain the relationship between hyperinsulinemia and SCI in hypertensive subjects. In previous studies of normal subjects (both younger and older) and patients with atherosclerotic disease, there was a positive association between insulin levels and these cardiovascular and hemostatic variables blood pressure, triglycerides and PAI-1) (3,4). In an in vivo study of rabbits, infusion of insulin or proinsulin increased plasma PAI-1 activity and gene PAI-1 expression in the aorta and liver (21).

Association of Hemostatic Abnormalities with SCIs

Among hemostatic risk factors for SCI in hypertensive patients, the plasma PAI-1 level was the strongest. In a previous report on hemostatic activation in clinically overt ischemic stroke, PAI-1 and t-PA levels were increased in both the early and late phases (22). According to the Oxfordshire Community Stroke Project classification, the PAI-1 and t-PA levels in patients with lacunar infarction were comparable to those of the patients with cortical infarction (22). In our study, the plasma PAI-1 level was negatively associated with the level of PIC (an indicator of plasmin generation), which tended to be negatively associated with the insulin profile. This suggests in vivo suppression of PAI-1 in plasmin generation, which is consistent with previous results found in healthy older subjects (4). When thrombin generation occurs in vivo, t-PA is simultaneously released to induce plasmin generation. This secondary fibrinolysis could act against increased thrombin generation. In the multiple infarct group, both thrombin-generation markers (F1+2 and TAT) were increased, but the PIC level was not increased, as compared with those levels in the noninfarct group. This imbalance between thrombin-generation and plasmin-generation may accelerate microatherothrombosis formation in small arteries, creating multiple lacunar infarcts, even in the silent stage of hypertensive cerebrovascular disease. In addition, t-PA in cerebrovascular endothelium may be related to the formation of a silent lacunar infarct. A recent study using immunohistochemistry and Western blotting disclosed that t-PA-containing vessels are mainly distributed in the smaller vessels (precapillary arterioles and postcapillary venules) in several stroke-prone regions of rat brains, which are often used to study the pathophysiologic consequences of cerebral ischemia (23). Thus, stroke-prone subjects may also be protected against high PAI-1 levels for developing small-vessel disease (lacunar infarct). However, once ECs in the small vessels are damaged due to aging or hypertension, or both, the effect of high plasma PAI-1 levels might be overt, as found in the present study.

We also found that higher plasma levels of vWF, which is synthesized and secreted from ECs, were associated with multiple SCIs. The vWF-mediated platelet adhesion to the injured endothelium is the first step in thrombus formation. The specific ELISA kit used in this study uses the monoclonal antibody to the functional epitope of vWF (12), whereas previous commercially available ELISA kits use the polyclonal antibody. Although various conditions such as EC damage, hypertension, diabetes, systemic atherosclerosis and inflammation (9,24) could increase plasma vWF levels, the increase in the functional vWF levels detected by this new ELISA kit may potentially lead to acceleration of microthrombus formation in multiple SCIs in elderly hypertensive subjects.

Risk Factors and Multiple SCIs

In the present study, there were no significant differences in hemostatic abnormalities between the low infarct group and the noninfarct group. The only significant difference was found in the multiple infarct group. Thus, hemostatic abnormalities may not be important in patients with only a few infarct formations, but these abnormalities may more often affect the larger-sized cerebral arteries, the branches of which end terminal cerebral arteries. In addition, multiple logistic analysis revealed that multiple infarcts were significantly independently associated with age, 24-h systolic blood pressure, vWF, PAI-1 and F1+2, but not with the insulin profile. Thus, hemostatic abnormalities may have some further impact, leading to the development of multiple lacunar infarcts, and may not be linked at all to hyperinsulinemia and insulin resistance.

Risk Factors and Location of SCIs

When we analyzed subtypes according to the location of SCI, we found that white matter infarcts were independently associated with age and 2-h insulin AUC, and basal ganglia infarcts were associated with PAI-1 and D-dimer. These results suggest that basal ganglia infarcts may be more closely related to systemic atherosclerosis and impaired hemostasis, because a recent report disclosed that the early stage of carotid atherosclerosis (intimal-medial thickening, as assessed by B-mode ultrasound) is positively associated with levels of PAI-1, t-PA and D-dimer in a population-based study (25). Thus, the progress of intracranial or extracranial atherosclerosis may explain the positive association between these fibrinolytic factors and SCI, especially in the basal ganglia, in older hypertensive subjects. In contrast, white matter infarcts may be more closely related to age-related arteriosclerosis of small perforating arteries. In addition, the 2-h insulin AUC was also independently associated with this subtype. Lipid profiles and smoking were related to neither subtype. However, in this study group, 28% of the patients had SCIs in both the white matter and basal ganglia. Thus, interpretation of the results is limited.

Study Limitations

The number of study subjects was relatively small, and this study was cross-sectional. In addition, there are racial and demographic differences in cardiovascular disease. Coronary artery disease is markedly less common, but stroke, particularly lacunar infarction, is slightly more common, in Japan than in Western countries. Thus, generalizability of our evidence found in elderly Japanese subjects (who continue the traditional Japanese lifestyle) should be tested in a larger, prospective study with subjects from different parts of the world.

Conclusions

In asymptomatic elderly hypertensive subjects, hyperinsulinemia appears to be associated with lacunar SCIs, particularly those located in the subcortical white matter. Hemostatic abnormalities showed an association with the presence of multiple lacunar infarcts, particularly those located in the basal ganglia.

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