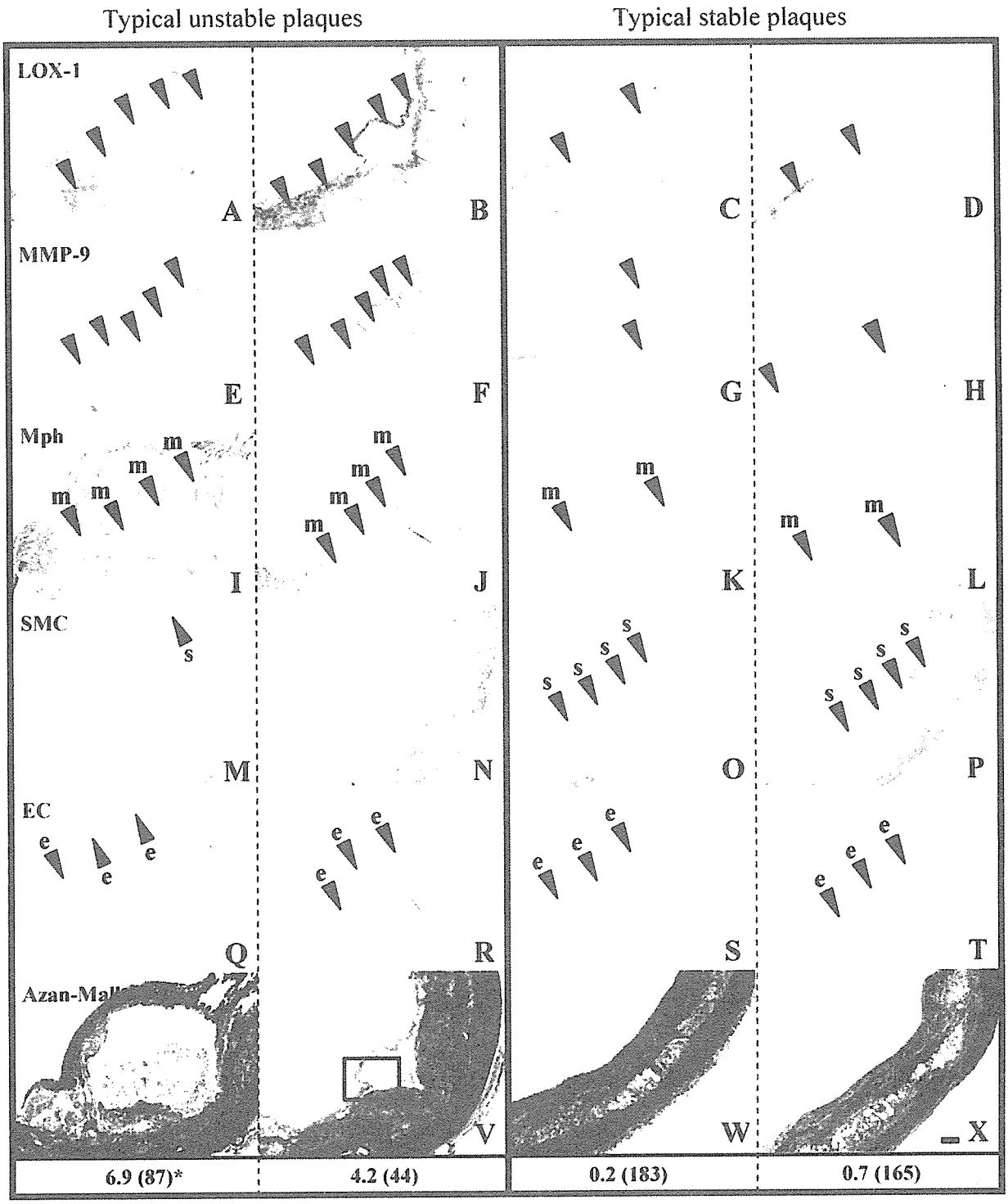

Fig. 2. Photomicrographs of atherosclerotic lesions in the thoracic aorta of WHHLMI rabbits showed typical unstable (left two columns) and stable (right two columns) atherosclerotic plaques. Immunohistochemical staining for LOX-1 (A to D), MMP-9 (E to H), macrophages (I to L), smooth muscle cells (M to P), and endothelial cells (Q to T) and Azan–Mallory staining (U to X). *The values in the lowest row show the plaque instability index (fibromuscular cap thickness) in each atherosclerotic plaque. Bar = 100 μm, magnification × 30. (m: macrophages; s: smooth muscle cells; e: endothelial cells).

3.2. LOX-1 expression and composition of plaques

The distribution of LOX-1 in atherosclerotic plaques was determined by immunohistochemical staining of serial sections with an anti-rabbit LOX-1 monoclonal antibody. As reported previously [18], LOX-1 was intensively expressed in the intima of atherosclerotic lesions, and endothelial cells and macrophages were stained positively for LOX-1

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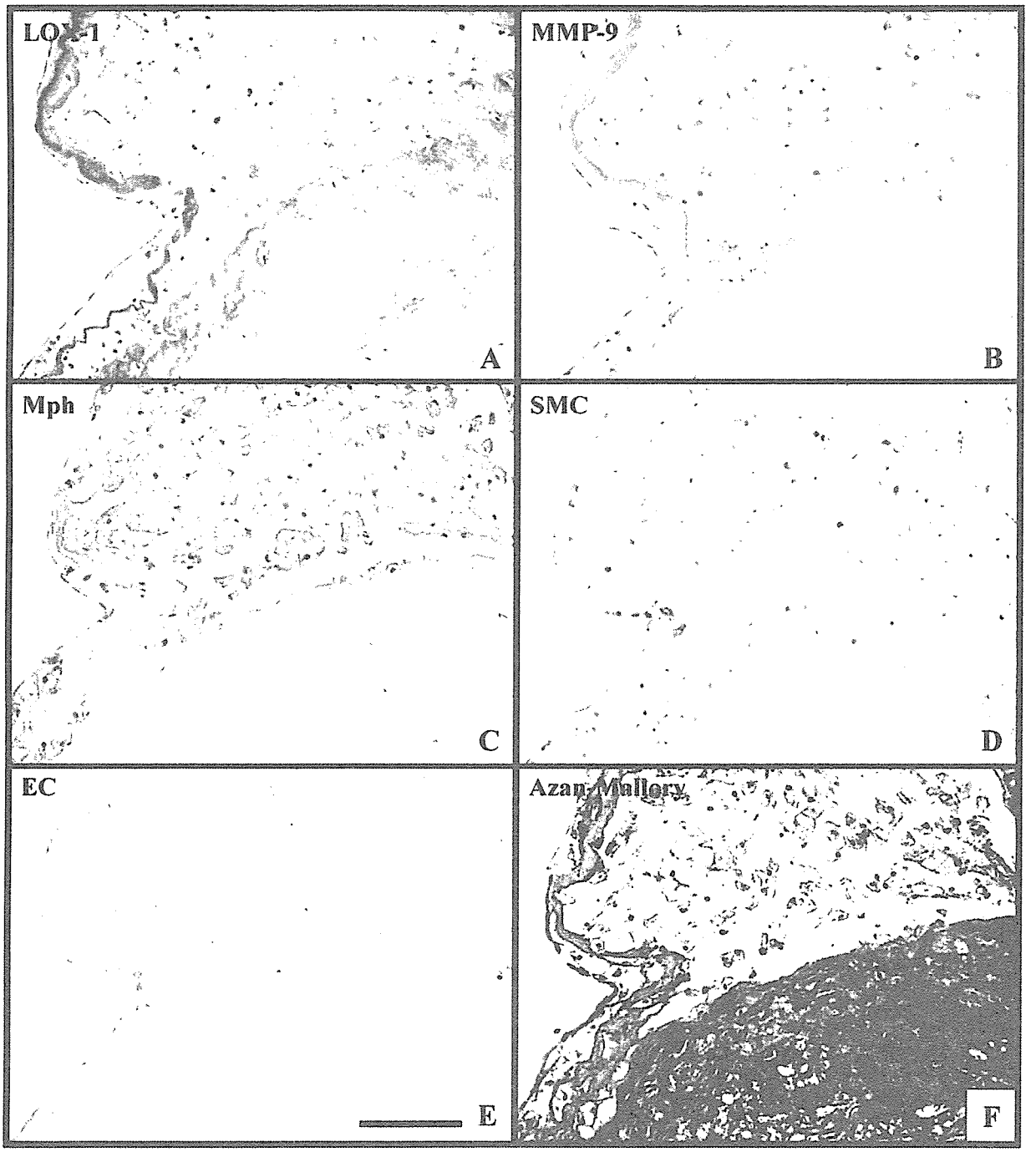

Fig. 3. Higher magnification images of unstable plaques corresponding to the area indicated as a square in Fig. 2 V. Immunohistochemical staining for LOX-1 (A), MMP-9 (B), macrophages (C), smooth muscle cells (D), and endothelial cells (E), and Azan–Mallory staining (F). Bar = 100 μm, magnification ×150.

(Fig. 2A–D, I–L, Q–T and 3A, C, E). In addition, intimal smooth muscle cells also prominently expressed LOX-1 (Figs. 2C, D, O, P and 3A, D) as previously reported in advanced human atherosclerotic plaques [19], although LOX-1 expression levels varied among atherosclerotic plaques (Fig. 2A–D). LOX-1 immunostaining was not observed in aortic sections from normocholesterolemic control rabbits (data not shown).

3.3. Correlation of LOX-1 expression density with plaque instability index or MMP-9 expression density

Using the histological images, we carried out quantification analyses as described in the Methods. LOX-1 expression density was positively correlated with the plaque instability index ($R = 0.74$, $P < 0.0001$, Fig. 4A) in this animal model of atherosclerosis. Furthermore, LOX-1 expression density

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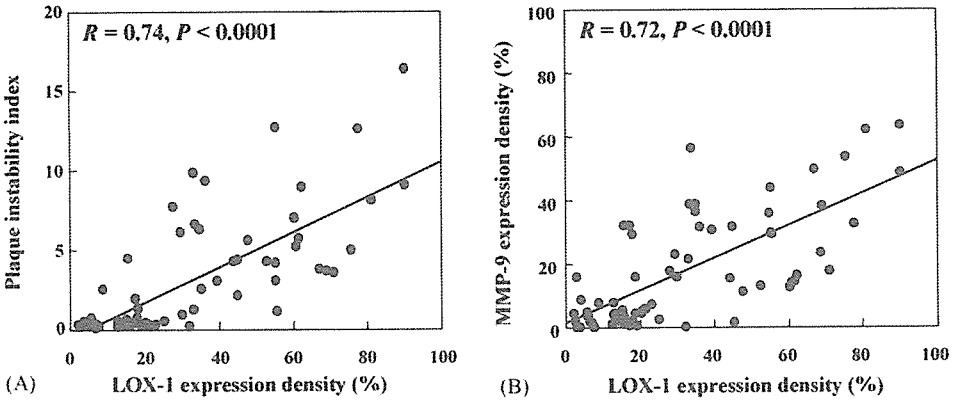

Fig. 4. Correlation of LOX-1 expression density with plaque instability index (A), or MMP-9 expression density (B), in atherosclerotic plaques in the thoracic aorta of WHHLMI rabbits ($n = 70$). Regression analyses demonstrated a positive correlation between LOX-1 expression density and plaque instability index ($R = 0.74$, $P < 0.0001$), as well as between LOX-1 expression density and MMP-9 expression density ($R = 0.72$, $P < 0.0001$).

positively correlated with MMP-9 expression density ($R = 0.72$, $P < 0.0001$, Fig. 4B), thus suggesting roles for LOX-1 in MMP expression, as well as apoptosis and foam cell transformation, as shown in cultured cells.

3.4. Classification of atherosclerotic plaques based on fibromuscular cap thickness

Atherosclerotic plaques were classified into two groups based on fibromuscular cap thickness (cut off value: 100 μm). Levels of LOX-1 expression density were significantly higher in the atherosclerotic plaques with thinner fibromuscular caps than those with thicker ones (51.1 ± 21.1 versus 17.1 ± 13.5 %, $P < 0.0001$).

3.5. LOX-1/MCP-1 expression and apoptotic events in atherosclerotic lesions

Fig. 5 shows LOX-1 and MCP-1 expression, and apoptotic events in unstable plaques. In the lipid core area, MCP-1 expression and TUNEL-positive nuclei were prominent mainly in macrophages (foam cells), in which LOX-1 expression was prominent. On the other hand, neither LOX-1 nor MCP-1 expression, nor TUNEL-positive cells were detectable in the fibromuscular cap area. Thus, LOX-1 expression appeared to be co-localized with MCP-1 expression and TUNEL-positive cells in macrophages (foam cells) located in the lipid core area. Neither MCP-1 expression nor TUNEL-positive nuclei were detectable in aortic sections from normocholesterolemic control rabbits (data not shown).

4. Discussion

We investigated the LOX-1 expression in association with histological markers of plaque instability, such as the plaque instability index, MMP-9 expression and fibromuscular cap thickness, and examined the co-localization of LOX-1 expression with MCP-1 expression and apoptotic events in hypercholesterolemic rabbits.

To evaluate the morphological destabilization of atherosclerotic plaques, we proposed a 'plaque instability index' calculated as the ratio between the two histological areas in atherosclerotic plaques: the lipid core area (macrophages plus extracellular lipid deposits) and the fibromuscular cap area (smooth muscle cells plus collagen fibers). As a histological index of atherosclerotic plaque, Shiomi et al. previously proposed a 'vulnerability index', calculated as the ratio between the two histological components (lipid and fibromuscular components) in the entire intima [21]. Compared to the vulnerability index, the plaque instability index would more directly reflect the thickness of the fibromuscular cap and the plaque instability because it was not calculated from the whole intimal area, but from the atherosclerotic plaque area. This plaque instability index was significantly correlated with LOX-1 expression density.

MMP-9, also known as gelatinase B or 92 kDa type IV collagenase, has been found to be prominently expressed in unstable atherosclerotic plaques in humans. Accordingly, MMP-9 has been suggested to be involved in the rupture of atherosclerotic plaques [23,24]. It has been reported that LOX-1-mediated uptake of Ox-LDL modulates the expression and activity of MMPs in cultured endothelial cells [13]. However, it remained unknown whether LOX-1 expression is associated with MMP-9 expression in vivo. This study showed, for the first time, that LOX-1 expression density was significantly correlated with MMP-9 expression density in atherosclerotic plaques. Thus, LOX-1 is also responsible for the Ox-LDL-induced expression of MMPs and thereby, the destabilization of plaques.

Because a fibromuscular cap thickness less than 100 μm has been reported as a marker of susceptibility to plaque rupture [25], we used this cut-off value to classify atherosclerotic plaques into two groups. According to this cut-off value, LOX-1 expression density was found to be significantly higher in the thinner fibromuscular cap group (<100 μm) than thicker cap group (>100 μm).

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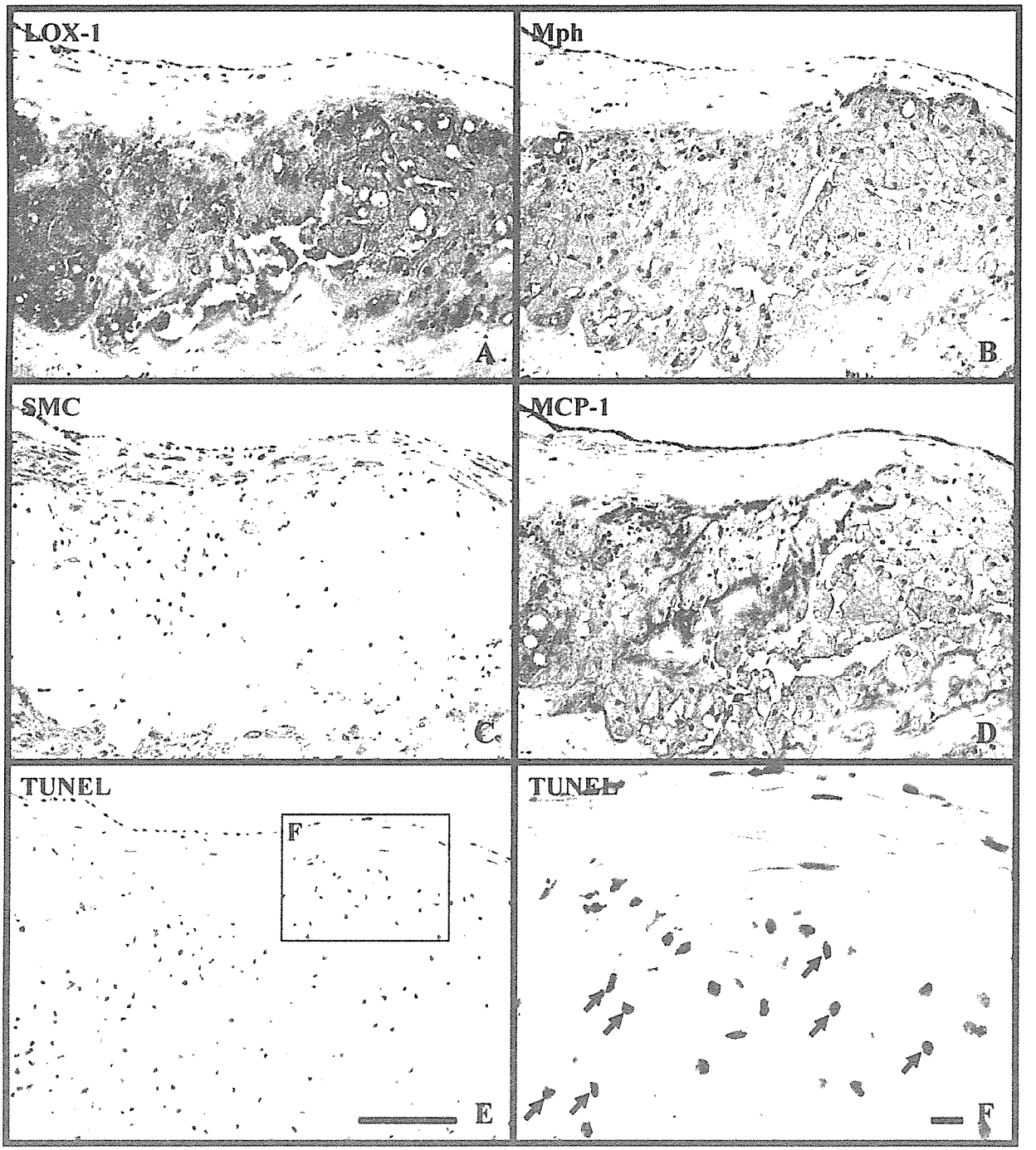

Fig. 5. Typical microscopic images of immunohistochemical staining for LOX-1 (A), macrophages (B), smooth muscle cells (C), and MCP-1 (D), and TUNEL staining (E) in the lipid core area of unstable plaque. Panel (F) showed a higher magnification image of the area indicated as a square in panel (E). Arrows indicate TUNEL-positive nuclei. Bar = 100 μm, magnifications ×150 (A–E), ×450 (F).

LOX-1 was reported to be expressed in endothelial cells and macrophages in early atherosclerotic lesions of aortas from 2-month-old WHHL rabbits [18]. In the present study, however, we used the aortas of mature rabbits to examine advanced atherosclerotic plaques. LOX-1 in these advanced plaques was colocalized with smooth muscle cells in fibromuscular caps, macrophages, endothelial cells and Azan-positive areas. Smooth muscle cells and macrophages in advanced atherosclerotic plaques in humans similarly express LOX-1 [19], thus indicating the histological similarity of atherosclerotic plaques between humans and this animal model. In this study, LOX-1 expression was also observed in Azan-positive areas. This might result from soluble LOX-1 bound to extracellular matrix proteins [26]. In

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destabilized plaques, smooth muscle cells might disappear by apoptosis after expressing abundant LOX-1 and releasing large amounts of soluble LOX-1.

We also compared LOX-1 expression with other two crucial factors in the destabilization of atherosclerotic plaques, such as MCP-1 expression and apoptotic events. MCP-1 appears to be a key molecule regulating atherosclerotic plaque instability by recruiting monocytes/macrophages into the plaques and thus eliciting proinflammatory responses. It has been reported that LOX-1-mediated uptake of Ox-LDL induces MCP-1 expression in cultured cells [7]; however, it remained unknown whether LOX-1 expression is associated with MCP-1 expression in vivo. In the present study, we provide evidence, for the first time, that LOX-1 expression is co-localized with MCP-1 in the lipid core area. Furthermore, TUNEL-positive apoptotic cells were also co-localized with LOX-1 expression in the lipid core area. Apoptosis of foam cells and macrophages is thought to contribute to the formation of the acellular lipid core [27]. Taken together, LOX-1 is suggested to be involved in atherosclerotic plaques rupture by regulating MCP-1 expression and apoptotic events, in addition to MMP expression.

Animal models suitable for studying the spontaneous rupture of unstable plaques have yet to be established. In this hypercholesterolemic rabbit model, the rupture of atherosclerotic plaques was reported to be undetectable in aortic lesions and most coronary lesions [28]. However, rupture-prone unstable plaques characterized in humans as those consisting of thin fibromuscular caps and large lipid cores with numerous macrophages were similarly observed in both aortic (Fig. 2) and coronary lesions in this animal model [28]. Atherosclerotic plaques with these histological characteristics appear to be causative of acute coronary syndrome in humans [4,29,30]. In the present study, therefore, we utilized atherosclerotic plaques consisting of fibromuscular caps and lipid cores in this rabbit aortas as a model to analyze plaque instability, focusing on the components of plaques and expression of LOX-1 and MMP-9. The relationship between LOX-1 expression and histological instability may also be observed in human coronary plaques, although this point should be clarified in the future. In fact, Ox-LDL, the ligand of LOX-1, has been shown to be more abundantly accumulated in coronary atherosclerotic plaques of patients with acute coronary syndrome than stable angina pectoris [31].

Aikawa et al. demonstrated the stabilization of atherosclerotic lesions resulting from lipid lowering on cessation of cholesterol-feeding and statin administration, using the aortas of rabbits with diet-induced hypercholesterolemia and ballon-injury [32], as well as genetically hypercholesterolemic WHHL rabbits [33]. In the present study, we did not include any interventions, such as lipid lowering or balloon injury; however, atherosclerotic plaques with a variety of histological characteristics were observed in the same individual WHHLMI rabbits. Therefore, we compared these histological characteristics with the expression of LOX-1 and MMP-9, both of which appear to be key molecules regulating plaque instability.

In addition, lipid lowering may also stabilize atherosclerotic plaques and may reduce LOX-1 expression in this model, as well as in humans, because lipid lowering can reduce the accumulation of Ox-LDL in arterial walls which induces LOX-1 expression [11]. Furthermore, statins directly suppress LOX-1 expression in cultured vascular cells [34]; therefore, statins can also reduce LOX-1 expression in vivo independently of Ox-LDL levels. These points, which could provide clinically important and intriguing insights, should be clarified in the future.

In summary, the present study demonstrated that LOX-1 expression is associated with the instability of atherosclerotic plaques in a hypercholesterolemic animal model. Thus, LOX-1 may be a therapeutic target to prevent the rupture of atherosclerotic plaques, since the serum level of soluble LOX-1 has recently been shown to have diagnostic value for acute coronary syndrome [35].

Acknowledgements

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Stroke-Independent Association Between Metabolic Syndrome and Functional Dependence, Depression, and Low Quality of Life in Elderly Community-Dwelling Brazilian People

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OBJECTIVES: Metabolic syndrome (Met.S) is a risk factor for stroke, dementia, and ischemic heart disease (IHD). It is unclear whether Met.S is an independent risk factor for functional dependence, depression, cognitive impairment, and low health-related quality of life (HRQoL) in a population free of clinical stroke.

DESIGN: Cross-sectional.

SETTING: Two communities in southern Brazil.

PARTICIPANTS: Four hundred twenty people aged 60 and older.

MEASUREMENTS: An adapted (body mass index $\geq$30 kg/m^2 and blood pressure $\geq$140/90) Adult Treatment Panel III definition was used in diagnosing Met.S. Depression (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Revised*) and Mini-Mental State Examination were evaluated along with activities of daily living (ADLs) and instrumental activities of daily living (IADLs). HRQoL was measured using a visual analogue scale (0–10). All values were adjusted for age, sex, and presence of IHD.

RESULTS: Forty (9.5%) subjects had a stroke and were excluded from the final analysis. Met.S was present in 37.4% of the stroke-free population. Met.S was significantly and independently associated with 2.24 times as much ADL dependence, 2.39 times as much IADL dependence, a 2.12 times higher risk of depression, a 2.27 times higher likelihood of cognitive impairment, and a 1.62 times higher chance of low self-perceived HRQoL (all $P<0.05$). Adjustment for its own components reduced the strength of the above associations but did not eliminate their statistical significance. If Met.S were removed from this population, dependence, depression, cognitive impairment, and low QoL would be reduced 15.0% to 21.4%.

CONCLUSION: Met.S was significantly associated with functional dependence, depression, cognitive impairment, and low HRQoL, and its effects were independent of clinical stroke, IHD, and its own individual components. J Am Geriatr Soc 55:374–382, 2007.

Key words: metabolic syndrome; functional dependence; depression; cognitive impairment; QoL

One of the most widely accepted theories of aging is the combined oxidative stress/protein-glycation theory, whereby accumulation of these effects over time leads to aging, age-associated diseases, neurofunctional dependence, cognitive impairment, frailty, and death.[1]

Metabolic syndrome (Met.S) consists of a cluster of obesity, glucose intolerance, hypertension, low high-density lipoprotein cholesterol (HDL-C), and high triglycerides; most of these have been shown to be risk factors for stroke and ischemic heart disease (IHD).[2] The pathophysiological unified basis of the syndrome seems to involve hyperinsulinemia,[3] which, in turn, is strongly associated with obesity.[4] Met.S itself has also been shown to be a risk factor for IHD,[5] stroke,[5–7] and dementia,[8] including Alzheimer's disease.[9] A recent study has found Met.S to be an independent risk factor for asymptomatic stroke.[10]

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The Met.S phenotype seems to be the consequence of chronic exposure to the effects of obesity,[4] sedentarism,[11] and an unhealthy diet[11] upon a susceptible genotype.[12] Obese individuals with Met.S may be considered the subfraction of obese individuals subjected to more oxidative stress and therefore manifesting to a higher degree the adverse metabolic consequences of obesity.[4] In addition to its usual association with obesity, Met.S seems to accelerate biological aging by promoting protein glycation,[1] insulin resistance, and telomere attrition.[13] In this sense, subjects with Met.S might represent a better population model of overfeeding[14] and accelerated aging (reverse of caloric restriction) than those with isolated obesity. In particular, Met.S might be a more consistent risk factor in older people, in whom obesity itself loses much of its risk.[15]

"Successful" aging might be defined as aging without major chronic, debilitating diseases and keeping functional independence and proper cognitive and affective neurofunctions to a maximum extent before death,[16,17] although in practice, in modern societies, "usual" aging is more often associated with debilitating diseases and progressive loss of autonomy, in which advanced cases the "usual" gives place to the clearly "pathological" cases.[16]

Many aging changes that have been interpreted as age-intrinsic have turned out to be "usual" only in modern societies (i.e., they were shown not to belong to the physiology of aging itself, as evidenced in primitive communities).[18] Met.S may be considered the modern society chronic syndrome epidemic par excellence, for it encompasses most of the main atherogenic risk factors for cardiovascular diseases.[19]

Functional dependence, cognitive impairment, and depression are central reasons that many people do not experience successful aging.[17] Many associations have been reported between the metabolic factors of Met.S (hyperinsulinism, obesity, glucose intolerance, low HDL-C, and hypertension) and features of pathological aging (functional dependence, cognitive impairment, and depression).[20–26]

Clinically manifested stroke is the most common condition responsible for functional decline in older people.[27] After stroke, IHD is one of the most important causes or correlates of functional dependence, cognitive impairment, and (vascular) depression in older people.[27] Met.S, in turn, is strongly associated with and predicts the occurrence of stroke[5–7] and IHD.[5]

Given these considerations, it would be expected that Met.S would be associated with functional dependence, cognitive impairment, depression, and low health-related quality of life (HRQoL), even in a stroke-free population, yet no comprehensive study could be found to document these relationships. It is also unclear whether Met.S is, independently of its individual components, associated with the above outcomes in stroke-free subjects and when controlling for IHD.

In this study, it was hypothesized that Met.S would be an independent determinant of functional decline, depression, cognitive impairment, and lower self-perceived health in stroke-free community-dwelling Brazilian older adults.

METHODS

Population and Setting

This study invited 450 older adults ($\geq$60) living in two towns ($\sim$ 30,000 inhabitants each) in the southernmost Brazilian state, Rio Grande do Sul. This randomized sample was selected from a list provided by the Department of Social Assistance of each town that contained virtually all people aged 60 and older in the town (n = 4,547 for both towns). Whenever an older person was identified, his or her spouse was also identified and, if older than 60, invited to participate in the research by the local health agent. Of the final sample of 422 (response rate = 93.8%), 238 (56.4%) were married, and 111 couples participated in the research (52.6%).

Brazil is a heterogeneous society, made up primarily of whites (53.4%) and Mestizos (40.4%) inhabitants.[28] Two towns, Estancia Velha and Charqueadas, were selected to better ethnically represent southern Brazilian older people. Estancia Velha has a predominantly white population, whereas Charqueadas' inhabitants are mainly Mestizo.[28] A preliminary analysis did not evidence any major differences in terms of prevalence of Met.S, functional dependence, depression, or average HRQoL between these two towns. Data were therefore pooled and analyzed together to account for a reliable sample of southern Brazilian older people.

Dependent individuals were brought to the research site and taken home in an appropriate vehicle.

Measurements

Trained (1 full day) sixth-year medical students performed the interviews and the battery of geriatric tests. At the end of the questionnaire, all subjects submitted to blood examination, a battery of geriatric assessment scales, and geriatric evaluation. The blood examinations consisted of analysis of fasting glucose, hemoglobin, albumin, total cholesterol, HDL-C, triglycerides, and creatinine levels.

For diagnosis of Met.S, an adapted form of the Adult Treatment Panel (ATP) III[2] definition using two of the World Health Organization (WHO)[29] criteria was applied for use in Brazilian older people, whereby body mass index (BMI) of 30 kg/m^2 and higher and blood pressure of 140/90 mmHg or higher were used to diagnose the obesity and hypertensive component of the syndrome, respectively. The WHO definition of Met.S does not allow Met.S to be treated as a continuous variable, as was done in this study (Figure 1). In addition, the ATP III definition uses waist circumference (WC) instead of BMI and adopts a criterion for normal blood pressure as systolic less than 130 and diastolic less than 85 mmHg, which is not as clear a "low risk" category as it is in young adults.[30] Therefore, a cutoff point of 140/90 mmHg was adopted to define hypertension in older people, as in the WHO Met.S definition.[29] In this study, BMI was used instead of WC for several reasons. WC is strongly ethnicity-specific,[31] and there is not yet a standard cutoff point for use in South American men and women; some studies, including one conducted in Brazil,[32] have shown that the WC criteria for Met.S does not significantly improve the prediction of Met.S-associated cardiovascular outcomes when compared with BMI of 30 kg/m^2 or greater; and WC was not evaluated in this population.

The diagnosis of Met.S, according to these modified ATP III criteria, required three or more of the following five criteria: obesity (BMI $\geq$30 kg/m^2), HDL-C less than 40 mg/dL in men or less than 50 mg/dL in women, triglycerides of 150 mg/dL or greater, blood pressure of 140 mmHg or greater for systolic or 90 mmHg or greater for diastolic, and fasting glucose of 110 mg/dL or greater (glucose intolerance plus frank diabetes mellitus). People without Met.S were the control group. In addition, according to the newest ATP definition,[2] the use of drugs for hypertension, high glucose and triglyceride, and low HDL-C was also considered to be a positive score for each respective Met.S component.

In addition, the WHO criteria for Met.S were used solely to compare its prevalence with the prevalence of the modified ATP III definition and to enable prevalence comparisons with reports from other countries. The WHO defines Met.S as the presence of diabetes mellitus or impaired glucose tolerance (as above) plus the presence of two or more obesity, hypertension (as above), and dyslipidemia (hypertriglyceridemia as above or HDL-C $<$40 mg/dL for women and $<$35 mg/dL for men).

Unless otherwise stated, Met.S refers to the modified ATP III criteria.

Mini-Mental State Examination (MMSE)[33] score was used to evaluate cognitive function. Cognitive impairment was defined as a MMSE score of 23 points or less. Depressive symptoms were assessed using the 15-item Geriatric Depression Scale (GDS), Brazilian Portuguese validated version.[34] A psychiatrist diagnosed depression according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Revised*.[35] Cases were screened using two questions: Have you dropped many of your activities and interests? Do you often feel sad or depressed? Cases with positive answers to either question were selected for the psychiatry interview. Major and minor (dysthymia) depression were considered as a single "depression" variable.

Functional status was assessed using an standardized questionnaire and included activities of daily living (ADLs),[36,37] instrumental activities of daily living (IADLs), and advanced (social and intellectual) ADLs (the last two constituting the Tokyo Metropolitan Institute of Gerontology (TMIG) scale).[38] Difficulty performing one or more of any of the ADLs was considered to be dependence for the respective ADL.

HRQoL was assessed using a 10-cm visual analogue scale,[39] ranging from 0 (worst possible score) to 10 (best). The median score was used as the cutpoint when categorizing into low and high score groups.

The validity and reproducibility of all of the above scales and methods have been well established; detailed methodologies being described elsewhere.[33–39]

Comorbidities were assessed using a standardized questionnaire and medical history. Diabetes mellitus type 2 was defined as fasting plasma glucose of 126 mg/dL or higher or current use of antidiabetic drugs.[40] Stroke diagnosis was performed on the basis of clinical history, findings on the neurological examination, and previous brain computed tomography (CT) scans. Paper medical charts from the local health unit were reviewed for confirmation in case of history of stroke without present neurological localization. Cases were entered as positive if at least one brain CT scan or magnetic resonance image scan confirming the stroke was registered.

IHD was defined as presence of angina pectoris, use of nitrates, history of myocardial infarction; positive ECG, effort-ECG, or coronary angiography; or history of coronary angioplasty or bypass. Heart disease was defined as any heart abnormality that might have been causing symptoms to the patient and included IHD, heart failure, and arrhythmias, as identified in the clinical history or medical examination.

Statistical Analysis

For statistical analyses, SPSS version 11.5 (SPSS Inc., Chicago, IL) was used. Multivariate logistic regression analysis was used to assess the relationships between categorical variables. Independent *t* test was used for comparisons between two groups and analysis of variance for comparisons between more than two groups. Analysis of covariance was used to adjust means to age, sex, and IHD. A 95% confidence interval (CI) was used and calculated on the basis of the binomial distribution. Except where otherwise stated, all values were adjusted for age, sex, and IHD.

Initial baseline analysis included stroke distributions (Table 1). In a second step, cases with stroke were removed from the analysis involving the relationship between Met.S, functional status, depression, cognitive impairment, and HRQoL (from Figure 1 and Table 2 on).

Population attributable risk (PAR) is defined as the fraction of total disease experience in the population that would not have occurred if the effect associated with the risk factor of interest had been removed.[41] To directly calculate the adjusted PAR, the Interactive Risk Assessment Program (IRAP) was used.[41] PAR was adjusted for age, sex, and presence of heart disease.

The ethics committee of the Catholic University of Rio Grande do Sul State, Brazil, approved this project. Informed consent was obtained from all participants. Surrogates were also asked to sign the informed consent form when subjects' MMSE scores were 23 points or less.

RESULTS

Of the 450 subjects initial invited, 422 (93.8%) participated in the research. Mean age was 68.3 (range 60–91), and women represented 63.3%. Met.S was present in 166 (39.3%) subjects according to the modified ATP III criteria and 152 (36.0%) individuals using the WHO criteria. Dependence in ADLs was present in 107 (25.4%) subjects, dependence in IADLs in 119 (28.2%), and dependence in advanced ADLs in 208 (49.3%). Depression was diagnosed in 72 (17.1%) subjects and cognitive impairment in 144 (34.1%).

Women were 2.96 times as likely to be dependent in ADLs, 4.23 times as likely to be dependent in IADLs, 2.29 times as likely to be depressed, and 2.48 times as likely to have cognitive impairment (all P = .006); women were not more likely to have Met.S (95% CI = 0.54–1.36) or to have a lower self-rating of their HRQoL (95% CI = 0.62–1.37). Moreover, the associations between Met.S and evaluated outcomes described below were not restricted to women, and associated risk was in general not notably dissimilar between the sexes (not shown).

Table 1. Baseline Characteristics According to the Presence or Absence of Metabolic Syndrome

Characteristic	Metabolic Syndrome		*P*-value*
	No n = 256 (60.7%)	Yes n = 166 (39.3%)	
Age, mean	68.5	68.1	.46
Female, n (%)	168 (65.6)	99 (59.6)	.18
White/Mestizo, %	54.8/45.2	55.2/44.8	.59
Monthly income, US$	712	662	.34
Education, years, mean	3.11	2.88	.20
Anemia, n (%)	66 (25.8)	35 (21.1)	.31
Albumin, mg/dL	4.23	4.28	.37
Systolic BP, mmHg	152.3	159.4	.003
Diastolic BP, mmHg	88	91	.004
Hypertension, n (%)	207 (80.9)	157 (94.6)	<.001
BMI, kg/m^2			
Mean	26.4	30.4	<.001
≥30.0 (obese)	33 (12.9)	143 (86.2)	<.001
High-density lipoprotein cholesterol, mg/dL			
Mean	48.9	34.8	<.001
<40 men; <50 women, n (%)	69 (27.0)	153 (92.2)	<.001
Triglycerides, mg/dL			
Mean	119.6	168.2	<.001
≥150, n (%)	22 (8.6)	115 (69.3)	<.001
Glucose			
Mg/dL, mean	110.7	144.1	<.001
Intolerance, n (%)	19 (7.4)	59 (35.6)	<.001
Diabetes mellitus type 2, n (%)	30 (11.7	73 (44.0)	<.001
Metabolic syndrome components, mean	1.48	3.52	<.001
Taking prescribed and regular drugs, n (%)	230 (89.8)	148 (89.2)	.79
Alcohol, ≥1/week	63 (24.6)	31 (18.7)	.29
Smoking, n (%)			
Past	61 (23.8)	43 (25.9)	.36
Present	32 (12.5)	20 (12.0)	.85
Bone fracture, n (%)	78 (30.5)	46 (27.7)	.57
Osteoarthrosis, n (%)	119 (46.5)	82 (49.4)	.55
Heart disease, n (%)	73 (28.5)	52 (31.3)	.60
Ischemic heart disease, n (%)	21 (8.2)	24 (14.5)	.119
Stroke, n (%)	18 (7.0)	22 (13.3)	.109
Actively working, n (%)†	171 (66.8)	91 (54.8)	.008
Regular exercise, n (%)‡	106 (41.4)	32 (19.3)	.003

* T test for numeric variables and chi-square test for categorical variables.
† Remunerated (mainly manual labor), part-time included.
‡ ≥three times a week.
††Because physical activity decreases the risk of obesity and improves insulin sensitivity independent of its effect upon body mass index (BMI),[42] adjusting for either of these two variables in Tables 2–4 would be considered overadjusting.
BP = blood pressure.

As expected, most metabolic-associated atherogenic risk factors were higher in the Met.S group (Table 1). Stroke was present in 40 individuals (9.5%). Stroke prevalence was higher in the Met.S group, but it not significantly so. Three hundred eighty-two individuals were free of stroke; 143 (37.4%) of these had Met.S.

Figure 1 illustrates significantly worse scores for all evaluated variables as the number of Met.S components increased in the stroke-free population ($P < .05$ for all variables).

Table 2 depicts the mean adjusted score for each applied test or scale according to the presence or absence of Met.S. Even though advanced ADL scores were lower in the Met.S group, these trends did not reach significance. For all other variables, mean scores were significantly lower in the Met.S group.

Table 3 shows the mean value or prevalence for several individual components of Met.S according to dependence in ADLs. After adjusting for age and IHD, women were 2.96 times as likely to be dependent in ADLs as men ($P < .001$). After adjusting for age, sex, and IHD, a one-digit increase in BMI (kg/m^2) increased the risk of being dependent in ADLs 7% ($P = .007$). Obesity was associated with a 1.91 times higher likelihood of dependence ($P = .004$). Ten

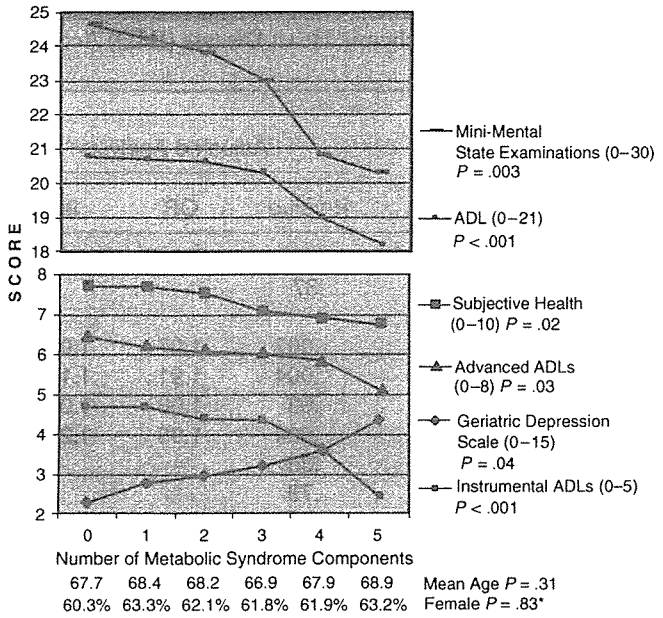

Figure 1. Cognitive, affective, and physical functions according to the number of Metabolic Syndrome components in the stroke-free population. *Chi-square test; analysis of variance for other *P*-values. ADL = activities of daily living.

mg/dL higher serum fasting glucose level was associated with a 1.6 times higher chance of dependence ($P = .03$), and diabetes mellitus increased the chance of dependence in ADLs by 1.98 times ($P = .004$).

Met.S was associated with a 2.24 (95% CI = 1.13–4.44; $P = .02$) greater chance of being dependent in ADLs. After controlling for all Met.S individual components, Met.S was still associated with a 1.71 times greater likelihood of being dependent in ADLs (95% CI = 1.02–2.87; $P = .04$).

Table 4 shows the CI, odds ratio (OR; if $P < .05$), and PAR of Met.S as an associated risk factor for low performance in the several evaluated neurofunctional variables and HRQoL. Met.S was significantly associated with greater dependence for all variables except advanced ADL scale. Met.S was significantly associated with a 2.27 times higher risk of cognitive deficit, a 2.12 times higher likelihood of depression, 2.24 times more dependence in ADLs, 2.39 times more dependence in IADLs, and 1.88 times higher odds for low HRQoL (all $P < 0.05$). Further adjustment for the individual components of Met.S reduced but did not eliminate the strength or significance of the association.

If Met.S were theoretically removed from the population (PAR), prevalence of cognitive impairment would decrease 21.3%, depression 20.1%, dependence in ADLs 20.5%, and dependence in IADLs 21.4%, and the number of people self-rating their HRQoL as low would decrease 15.0% (all $P = .05$).

DISCUSSION

Even after adjusting for IHD and individual components of Met.S, the Met.S construct was still independently associated with 1.58 to 2.02 greater odds for dependence in ADLs, IADLs, cognitive impairment, depression, and low HRQoL. This phenomenon suggests that Met.S, in addition to its unified hyperinsulinemic pathophysiological process, is also a valid clinical construct in geriatrics, because it represents a useful concept of risk.

As expected, regular practice of exercise and being actively working (mainly manual labor in this population) were negatively associated with Met.S (both $P < .01$), although because physical activity decreases the risk of obesity and improves insulin sensitivity independent of its effect on BMI,[42] adjusting for either of these variables would be considered overadjusting. For this reason, values in Tables 1–4 were not adjusted for either of these two variables.

Obesity was associated only with dependence in ADLs, whereas glucose intolerance (including frank diabetes mellitus) was associated with dependence in ADLs and cognitive impairment. Low HDL-C was associated only with low self-perceived health status. It has been previously reported that, in older people, low HDL-C is associated with low functional status,[43] but the finding that low

Table 2. Cognitive, Affective, Functional, and Health-Related Quality of Life (HRQoL) Mean Scores According to the Presence or Absence of Metabolic Syndrome in the Stroke-Free Population

	Metabolic Syndrome		
	No n = 239 (62.6%)	Yes n = 143 (37.4%)	
Cognitive, Affective, Functional, and HRQoL Scales	Mean ± Standard Deviation		*P*-value*
Geriatric Depression Scale score (0–15)	2.77 ± 2.8	3.79 ± 3.1	.04
Mini-Mental State Examination score (0–30)	24.15 ± 4.4	22.09 ± 4.9	.01
ADL scale score (0–21)	20.51 ± 1.5	18.78 ± 2.5	<.001
Instrumental ADL scale score (0–5)	11.19 ± 1.2	10.49 ± 1.5	<.001
Social ADL score (0–4)	3.60 ± 0.79	3.32 ± 0.89	.07
Intellectual ADL score (0–4)	2.66 ± 1.3	2.26 ± 1.2	.09
Tokyo Metropolitan Institute of Gerontology scale score (0–13)	10.67 ± 2.5	8.89 ± 2.9	<.001
HRQoL score (0–10)	7.46 ± 2.4	6.59 ± 2.4	.03

Note: Mean scores were adjusted for age, sex, and the presence of ischemic heart disease.
* Analysis of covariance.
ADL = activity of daily living.

Table 3. Metabolic Syndrome (Met.S), Its Individual Components, and Presence of Ischemic Heart Disease (IHD) According to Activity of Daily Living (ADL) Dependence Status in the Stroke-Free Population

Characteristic	ADL		Adjusted Analysis*		
	Independent n = 295 (77.2%)	Dependent n = 87 (22.8%)	P-value	OR	95% CI
Female, n (%)	173 (58.6)	72 (82.8)	<.001	2.96	1.80–4.87
Age, mean	68.1	68.5	.37	—	—
Body mass index, kg/m^2					
Mean	27.5	29.11	.007	1.07	1.02–1.12
≥30.0 (obese), n (%)	77 (26.1)	32 (36.8)	.004	1.91	1.17–3.12
Fasting glucose, mg/dL, mean	120.8	134.7	.03	1.06	1.01–1.11
Diabetes mellitus type 2, n (%)	64 (21.7)	30 (34.5)	.004	1.98	1.29–3.04
Systolic BP, mmHg, mean	153.1	153.7	.36	—	—
Diastolic BP, mmHg, mean	86.4	86.5	.79	—	—
Hypertension (BP 140/90), n (%)	253 (85.8)	76 (87.4)	.27	—	—
High-density lipoprotein, mg/dL					
Mean	43.2	43.6	.44	—	—
<40 men, <50 women, n (%)	150 (50.8)	45 (51.7)	.71	—	—
Triglycerides, mg/dL					
Mean	137.9	140.4	.32	—	—
≥150, n (%)	92 (31.2)	26 (29.9)	.46	—	—
Met.S Adult Treatment Panel III score, n (%)	110 (37.3)	45 (51.7)	.02	2.24	1.13–4.44
Number of Met.S components, mean	1.63	2.13	.001	1.42	1.16–1.74
IHD, n (%)	26 (8.2)	14 (13.2)	.13	1.45	0.69–2.96

* Logistic regression, adjusted for age, sex, and presence of IHD. For numeric variables, odds ratio (OR) and 95% confidence interval (CI) correspond to a change in 1 unit in each respective variable.
BP = blood pressure.

HDL-C is associated with low self-perceived health independently of functional status seems to be a new finding. Because both extremes of BMI tend to have low HDL-C, and frail older people tend to be underweight, those with a BMI less than 20 kg/m^2 were excluded, but this did not modify the strength of the above association.

Hypertension was not associated with any evaluated outcome. Patients with systolic heart failure and dementia tend to have lower blood pressure,[44] although after excluding patients with heart failure and MMSE scores less than 24, systolic blood pressure was negatively correlated with MMSE (correlation coefficient = –0.12; P = .048) but not with other evaluated variables (not shown).

As for functional dependence, the findings were in accord with those of the Rotterdam Study,[45] in which diabetes mellitus and overweight, but not hypertension, were cross-sectionally associated with a 1.5 to 2.0 times higher chance of locomotor disability. Hypertriglyceridemia was another Met.S component that was not associated with any of the evaluated outcomes.

Mean advanced (intellectual and social) ADL score had a significant tendency to worsen with increasing number of Met.S components (Figure 1), although lower mean advanced ADL score in those with Met.S was just of borderline significance (Table 2) and was not significantly associated with Met.S in the logistic regression (Table 4). These findings are in accordance with results from a study conduced in Japan in which the IADL subdimension of the TMIG scale was more consistently associated with hypertension and diabetes mellitus than the intellectual and social dimensions of this scale were.[46]

The concept of Met.S was the only variable that showed a consistent association with dependence in ADLs and IADLs, cognitive impairment, depression, and low HRQoL. This suggests that the metabolic alterations of Met.S itself (rather than its obesity component alone) promote pathological aging, physical dependence, depression, cognitive impairment, and decreased HRQoL.

Possible Mediative Mechanisms

Several pathophysiological factors might mediate the associations between Met.S, functional dependence, cognitive impairment, and depression found in this study. On a population level, peripheral arterial disease is just minimally associated with increased attributable risk for functional dependence (2.5%)[27] and would hardly explain the approximately 21% to 22% PAR for ADLs and IADLs associated with Met.S in this study. Hyperglycemia has been associated with general weakness, muscle cramps, blurred vision, and dizziness.[57] Decreased proprioception due to peripheral neuropathy may also bring dependence.[47]

Nevertheless, except for small-vessel disease, none of the above causes can explain the association between Met.S, cognitive impairment, and depression.[48] Moreover, ORs for cognitive impairment, depression, and functional dependence (ADL and IADL) were all strikingly similar (2.12–2.39), suggesting a common pathophysiological mediator process. Indeed, the three above neurofunctional outcomes were strongly associated between themselves, even after adjusting for age and sex (OR = 2.45–4.26; all

Table 4. Metabolic Syndrome (Met.S) and Its Individual Components as Associated Factors for Low Performance in Several (Neuro)Functional Variables and Low Health-Related Quality of Life (HRQoL) in the Stroke-Free Population

Met.S Components	Cognitive Impairment	Depression	Dependence in			Low HRQoL
			ADLs	Instrumental ADLs	Advanced ADLs	
			Odds Ratio (95% Confidence Interval)			
Obesity	1.13 (0.72–1.78)	1.20 (0.55–2.60)	1.93 (1.20–3.01)*	1.17 (0.73–1.88)	0.80 (0.52–1.23)	0.98 (0.64–1.50)
Hypertension	0.80 (0.70–1.63)	1.04 (0.69–2.27)	1.25 (0.63–2.47)	1.77 (0.93–3.35)	1.10 (0.62–1.94)	0.85 (0.48–1.51)
Diabetes mellitus or glucose intolerance	1.56 (1.03–2.26)*	1.33 (0.85–2.08)	2.03 (1.25–3.3)*	1.39 (0.91–2.13)	1.09 (0.70–1.71)	1.27 (0.81–1.99)
High-density lipoprotein cholesterol, mg/dL, <40 (men) or <50 (women)	1.28 (0.88–1.85)	1.17 (0.73–1.87)	1.16 (0.72–1.87)	1.47 (0.95–2.28)	1.14 (0.71–1.60)	1.87 (1.24–2.83)*
Triglycerides ≥150 mg/dL	1.03 (0.71–1.49)	0.97 (0.61–1.54)	1.24 (0.77–2.00)	1.05 (0.71–1.55)	0.91 (0.60–1.39)	1.39 (0.91–2.12)
Met.S†	2.27 (1.21–4.26)*	2.12 (1.05–4.28)*	2.24 (1.13–4.44)*	2.39 (1.20–4.76)*	1.34 (0.81–2.23)	1.88 (1.14–3.10)*
Met.S adjusted for components‡	1.82 (1.06–3.12)*	2.02 (1.11–3.68)*	1.71 (1.04–2.81)*	1.58 (0.99–2.52)*	1.70 (0.89–3.25)	1.59 (1.02–2.48)*
Met.S population attributable risk, %†	21.3	20.1	20.5	21.4	—	15.0

Logistic regression: adjusted for age, sex, and presence of ischemic heart disease.
* $P < .05$.
† Calculated from odds ratio (OR) adjusted for age, sex, and IHD but not Met.S components.
‡ Further adjusted for all Met.S individual components.
ADLs = activities of daily living.

$P = .002$; not shown), suggesting that they may belong to a single syndromic entity.

Mean physical and cognitive functions decline steeply after the seventh decade of life, but this decline is heterogeneous.[49] Small-vessel disease might be one of the pathological hallmarks of this transition and might also explain its heterogeneous character.[49] Met.S and hyperinsulinism are preferentially associated with cerebral microangiopathy.[20] Indeed, Met.S seems also to potentiate age-related leukoaraiosis,[20] which has been reported to be associated with frontal-subcortical lacunar strokes and selective cognitive, affective, and neuromotor dysfunctions.[48] Together, these neurofunctional abnormalities constitute what has been considered a new geriatric nosological entity, namely the frontal-subcortical (ischemic) geriatric syndrome.[48] Frontal-subcortical dysfunction may be a key point in explaining the concomitant and interrelated decline in cognitive, affective, and neuromotor functions in older people.[48]

A study of identical elderly male twins showed that the most significant determinant of late-life white-matter lesions were glucose levels, HDL-C, and systolic blood pressure, all of which are Met.S components.[26] Moreover, insulin levels are significantly higher in patients with lacunar stroke, subcortical atherosclerotic encephalopathy, and microangiopathy than in normal control subjects.[20]

Small-vessel disease and clinical stroke are involved in the etiology of cognitive impairment in older people.[49] In this study, Met.S was more strongly associated with cognitive impairment in the stroke-free population (OR = 2.27; $P < .01$) than with stroke in the original population (OR = 1.5; $P = .19$). These findings suggest that Met.S might be more associated with features of small-vessel cerebrovascular disease than with clinical stroke. In fact, Met.S (but not its conventional risk factors) was recently shown to be independently associated with intracranial atherosclerosis and lacunar (often silent) stroke.[7]

Met.S might have increased the risk of depression simply by promoting more functional dependence, although even after adjusting for functional status, Met.S was still significantly associated with a 1.53 (1.03–2.27) higher chance of having depression. This result points to a straightforward effect of Met.S on depression and suggests that its influence on the brain directly mediates this effect.[48] In addition to promoting stroke and cerebral small-vessel disease, Met.S seems also to accelerate age-associated loss of serotoninergic innervation and responsivity, a phenomenon associated with higher risk of depression.[21,22]

Finally, Met.S might lead to decreased neuromotor and cognitive functions via an accelerated biological neuroaging process itself.[4,13]

Limitations

This study has several limitations. Because epidemiological studies, especially those of cross-sectional design such as this one, cannot prove cause and effect when the end-point is an outcome of a chronic noncommunicable condition, findings from this study can be cited only as being consistent with the hypothesis in question.

Because present-diagnosed Met.S was independently associated with most evaluated neurofunctional variables, hyperinsulinism and other Met.S components are probably

still acting in synergy to promote vascular disease at older age. However, due to the cross-sectional nature of this research, these values might account for just a fraction of all the cumulative variance on neurofunctional decline attributable to Met.S. For a given cardiovascular risk factor, the maximum explanatory variance upon outcomes might be found some 10 to 20 years, or even more, before this outcome; this rule is also valid for the brain.[26] This phenomenon may account, at least in part, for the lack of association between hypertension and any of the evaluated outcomes in this study.

Final Remarks

The above results are consistent with a growing body of evidence that links obesity, Met.S, vascular disease, and subclinical inflammation to cognitive, affective, neuromotor, and functional decline.[20–36,46] The relationship between insulin resistance, cerebrovascular and neural aging is tantalizing in its potential to offer an integrated model for aging of the body and the brain.[24]

To the authors' knowledge, this is the first study to comprehensively show that Met.S is associated with functional dependence, cognitive impairment, depression, and low HRQoL in older people. Moreover, it also suggests that Met.S may be a risk factor for the above outcomes independent of its own individual components (which might act in synergy), stroke, and IHD. It also demonstrated that, if Met.S were theoretically removed from this population, dependence in ADLs and IADLs—and the prevalence of cognitive impairment, depression, and low HRQoL—would decrease 15.0% to 21.4%. In addition, this study also suggests that cognitive and functional decline and greater depressive symptomatology may be part of the same syndromic process by which Met.S atherogenic factors might be acting.

Recognition of Met.S as a risk factor not just for cerebrovascular disease, but also for "unsuccessful" aging would encourage the identification of this multirisk-factor condition and promote lifestyle modifications that would reduce all of the Met.S risk factors simultaneously. Because Met.S is a potentially reversible syndrome, once excess weight is lost and physical activity initiated (its ultimate causes), older people with Met.S should be treated aggressively.

CONCLUSION

Met.S was significantly and independently associated with 2.2 to 2.4 times more physical dependence in ADLs and IADLs, 2.3 times higher odds for cognitive impairment, a 2.1 times higher risk of coexisting depression, and a 1.9 times higher chance of low HRQoL. If Met.S were theoretically removed from this population, the above outcomes would be reduced 15.0% to 21.4%. Met.S might be a major determinant of functional dependence, cognitive impairment, depression, and low HRQoL in later life.

Preventing and treating Met.S may be an important step in "preventing senility" and promoting successful aging.

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Adiponectin and inflammatory markers in peripheral arterial occlusive disease

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Abstract

This study was designed to examine the plasma levels of adiponectin as well as markers of inflammation and endothelial function in peripheral arterial occlusive disease (PAOD), and to investigate the pathophysiological significance of adiponectin in this disease. Eighty-eight subjects with ($n=40$) and without PAOD ($n=48$) were enrolled. Multiple regression analysis including age, sex, body mass index, hypertension, diabetes, triglycerides, high-density lipoprotein cholesterol, creatinine, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cellular adhesion molecules-1 (sVCAM-1), von Willebrand factor, and high-sensitive C reactive protein (Hs-CRP) showed that adiponectin concentration was significantly lower in PAOD subjects (PAOD: 7.9 ± 0.7 μg/mL versus without PAOD: 9.5 ± 0.6 μg/mL, $F=4.94$, $p<0.03$). Furthermore, concentrations of adiponectin ($F=8.5$, $p<0.01$) as well as sICAM-1 ($F=5.8$, $p<0.02$), sVCAM-1 ($F=5.9$, $p<0.02$), and Hs-CRP ($F=3.8$, $p=0.05$) were independently associated with ankle–brachial index. In 27 subjects (10 with PAOD and 17 without PAOD), adiponectin levels in the femoral artery and saphenous vein were measured. A significant step-up of adiponectin from the artery to the vein was observed in subjects without PAOD (+13.0%, $p<0.01$), but not in subjects with PAOD (+0.4%, NS). Plasma adiponectin as well as Hs-CRP were followed before and after percutaneous transluminal angioplasty (PTA) in eight patients. Adiponectin showed a tendency to decrease after PTA (day 6, −30.6%), although Hs-CRP significantly increased. Adiponectin is decreased in patients with PAOD in proportion to the severity of the disease. Adiponectin concentration could be a marker of the existence of atherosclerosis, and measurement of its concentration may be helpful in assessment of the progress of atherosclerosis.

Keywords: Adiponectin; Inflammatory markers; Peripheral arterial disease; Atherosclerosis; Angioplasty

1. Introduction

Peripheral arterial occlusive disease (PAOD) usually occurs in male subjects over 40 years old, especially in individuals with hypertension, diabetes mellitus, hyperlipidemia, and cigarette smoking. Patients with PAOD have not only clinical discomfort, e.g., intermittent claudication and pain in their lower extremities, but also a high incidence of other atherosclerotic lesions and cardiovascular events [1]. Furthermore, it is now recognized that low-grade inflammation contributes importantly to the initiation and progression of vascular atherosclerotic lesions [2], and in fact an elevated level of C-reactive protein (CRP) is shown to be related to the development of PAOD [3].

At the early stage of atherosclerosis, endothelial cell activation by various inflammatory stimuli results in the synthesis of adhesion molecules and increases the adherence

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of monocytes [4]. The process of leukocyte adhesion and transendothelial migration are mediated by cellular adhesion molecules (CAMs), which are expressed on the endothelial surface in response to a variety of atherogenic stimuli [5,6]. Intercellular CAM-1 (ICAM-1) and vascular CAM-1 (VCAM-1) are two prototypic members of the immunoglobulin superfamily of CAMs that are important in focal leukocyte accumulation in subendothelial regions of atheroma [7], and elevated plasma levels of soluble forms of these molecules have been reported to be associated with atherosclerosis [8–10]. In addition, von Willeband factor (vWF) is one of the major hemostatic regulatory molecules synthesized by endothelium and plays a role in platelet adhesion and thrombus formation [11]. The plasma level of this molecule has been shown to increase with endothelial damage, suggesting that this could be a reliable marker of endothelial dysfunction [12].

Adiponectin is an adipose tissue-specific collagen-like factor, which is abundantly present in plasma, and was previously reported to be associated with lipid metabolism and insulin resistance. Adiponectin is one of the key molecules in the metabolic syndrome, and decreased plasma level of this molecule is associated with obesity [13], type-2 diabetes [14], and hypertension [15]. Furthermore, adiponectin attenuates the endothelial inflammatory response in vitro, and its concentration is decreased in patients with coronary artery disease [14,16,17]. A previous study also demonstrated that physiological concentrations of adiponectin had significant inhibitory effects on tumor necrosis factor-α-induced adhesion molecule expression in a dose-dependent manner in vitro [16]. These findings indicate that adiponectin acts as an endogenous anti-atherogenic factor, suggesting that plasma adiponectin may be helpful in preventing the development of atherosclerotic vascular diseases.

In this study, we examined the plasma adiponectin concentration and several markers of inflammation and endothelial function in PAOD, and evaluated the clinical significance of plasma adiponectin in atherosclerosis.

2. Subjects and methods

2.1. Study 1: Association between PAOD and plasma adiponectin concentration

A total of 88 subjects were selected from patients who were admitted and underwent medical investigation at the National Cardiovascular Center in Osaka, Japan. The numbers of subjects with and without PAOD (Control) were 40 and 48, respectively. Control subjects whose age, sex, and body mass index were not significantly different from those in PAOD subjects were recruited. PAOD was defined based on clinical symptoms, ankle–brachial index (ABI) of <0.90 [18], findings of magnetic resonance angiography and/or findings of aortic angiography. The subjects were classified into five groups according to Fontaine's classification (stage 0: no symptom; stage 1: feeling of cold in the lower legs; stage 2: intermittent claudication; stage 3; leg pain at rest; stage 4: ulcer caused by ischemia). However, subjects with Fontaine's stage 4 were excluded from this study, because local inflammation of the leg strongly affects serum levels of several inflammatory parameters. Diabetes mellitus was defined according to World Health Organization criteria [19]. Hypertension was defined as a systolic blood pressure of ≥140 mmHg and/or a diastolic blood pressure of ≥90 mmHg on repeated measurements, or receiving antihypertensive treatment. Ischemic heart disease was defined as a 75% or greater organic stenosis of at least one major coronary artery as confirmed by coronary angiography, or a history of myocardial infarction or percutaneous transluminal coronary angioplasty. Smoking was defined as current smoking or having a history of habitual smoking. Subjects with chronic renal failure, acute coronary syndrome, cardiogenic shock, nephrotic syndrome, overt congestive heart failure, or valvular heart disease were excluded. Furthermore, no subjects receiving steroid therapy were included in this study. All subjects enrolled in this study were Japanese, and gave written informed consent to participate in this study. All procedures of the present study were carried out in accordance with institutional and national ethical guidelines for human studies.

After overnight fasting, the subjects rested supine for 5 min and blood pressure was measured. A hand-held Doppler probe (Nicolet Vascular Pocket Dop II) was used to measure systolic pressures in the right brachial artery, right dorsalis pedis and posterior tibial arteries, left dorsalis pedis and posterior tibial arteries, and left brachial artery. Pressures were measured twice, in the order listed and then in reverse order. ABI was calculated by dividing the average pressure in each leg by the average of the four brachial pressures [20]. Average brachial pressure in the arm with the higher pressure was used when one brachial pressure was higher than the opposite brachial pressure in both sets of measurements, and the two brachial pressures differed by ≥10 mmHg in at least one set of measurements. The lowest leg ABI was used in all analyses. After blood pressure measurements, venous blood was drawn from an antecubital vein, and immediately transferred into ice-chilled glass tubes containing disodium EDTA (1 mg/mL) and aprotinin (500 U/mL) and centrifuged for 10 min at 4 °C. Plasma samples were frozen and stored at −80 °C until assayed. Height and body weight were measured and body mass index (BMI) was calculated. Insulin sensitivity was estimated using the homeostatic model assessment (HOMA) index; i.e., plasma glucose level × (plasma insulin level/22.5). Plasma concentration of adiponectin was determined by a sandwich ELISA system (Adiponectin ELISA Kit, Otsuka Pharmaceutical Co. Ltd.) as previously reported [13]. Plasma concentrations of soluble ICAM-1 (sICAM-1), soluble VCAM-1 (sVCAM-1), and vWF were measured using commercially available enzyme-linked immunosorbent assay kits (sICAM-1 and sVCAM-1, R&D Systems, Minneapolis, MN; vWF, AssayPro, Winfield,

MO). High sensitive CRP (Hs-CRP) was measured by nephelometry (SRL, Tokyo, Japan).

The following parameters were also determined: hemoglobin, hematocrit, total cholesterol, triglycerides, high-density lipoprotein (HDL-) cholesterol, and serum creatinine levels.

2.2. Study 2: Analysis of plasma adiponectin concentration in femoral artery and saphenous vein

Twenty-seven subjects (10 with PAOD and 17 without PAOD) were enrolled in this study. Diagnosis of PAOD and Fontaine's classification were performed as described above. Blood samples were drawn simultaneously from the femoral artery and the saphenous vein in each subject. In subjects with PAOD, blood was drawn from the leg with the lower ankle–brachial index. The exclusion criteria for this study were identical to those described above. Informed consent was also obtained from the subjects prior to their participation in this study.

2.3. Study 3: Plasma adiponectin concentration before and after percutaneous transluminal angioplasty (PTA)

Eight male PAOD subjects (age, 71.1 ± 2.0 years; BMI, 21.4 ± 1.3 kg/m^2; ABI, 0.75 ± 0.06; Fontaine's stage, 1.9 ± 0.1) were enrolled in this study. Before and 2 and 6 days after PTA, blood samplings were performed from the antecubital vein. The exclusion criteria of this study were identical to those previously described, and informed consent was also obtained prior to their participation in this study.

2.4. Statistical analysis

Means or proportions of clinical characteristics and cardiovascular risk factors were computed for each pattern. Continuous variables were expressed as mean ± S.E.M. Unpaired *t*-test was used to examine the differences in adiponectin between two groups. Pearson's correlation coefficients were used to assess the relations between adiponectin and all other variables. Multiple regression models were used to assess the relationship between adiponectin concentration and PAOD after adjustment for potential confounding factors. Multiple regression analysis was also carried out to examine independent associations of adiponectin, sICAM-1, sVCAM-1, vWF, and Hs-CRP with ABI. The significance of difference in plasma adiponectin concentrations between the femoral artery and saphenous vein was evaluated using paired *t*-test. The significance of differences in plasma adiponectin and Hs-CRP before and after PTA was evaluated using repeated-measures ANOVA.

A *p*-value less than 0.05 was considered statistically significant. All calculations were performed using a standard statistical package (JMP 4.0, SAS Institute, Cary, NC).

Table 1
Clinical variables in subjects with and without PAOD in Study 1

Variables	Control, $n = 48$	PAOD, $n = 40$
Age (years)	69.1 ± 1.1	69.8 ± 1.2
Sex (male/female)	37/11	32/8
BMI (kg/m^2)	23.1 ± 0.4	22.5 ± 0.4
Smoking (%)	77.1	87.5
IHD (%)	47.9	47.5
Diabetes (%)	37.5	45.0
Hypertension (%)	77.1	82.5
Adiponectin (μg/mL)	8.30 ± 0.59	5.52 ± 0.64$^{\#}$
sICAM-1 (ng/mL)	181.0 ± 7.8	207.7 ± 8.6*
sVCAM-1 (ng/mL)	405.4 ± 24.2	482.6 ± 26.5*
vWF (mU/mL)	317.6 ± 26.5	355.1 ± 29.0
Hs-CRP (mg/L)	1.2 ± 0.3	1.9 ± 0.3
Hemoglobin (g/L)	134.6 ± 2.8	135.9 ± 2.4
Hematocrit	0.405 ± 0.008	0.409 ± 0.007
Systolic BP (mmHg)	133.9 ± 2.5	137.8 ± 2.8
Diastolic BP (mmHg)	72.9 ± 1.5	72.7 ± 1.6
Total cholesterol (mmol/L)	5.031 ± 0.104	4.943 ± 0.114
Triglycerides (mmol/L)	1.174 ± 0.081	1.305 ± 0.088
HDL-cholesterol (mmol/L)	1.312 ± 0.052	1.137 ± 0.055*
HOMA	1.22 ± 0.20	1.73 ± 0.21
Serum creatinine (μmol/L)	83.61 ± 3.70	83.98 ± 4.05
Ankle–brachial index	1.08 ± 0.02	0.61 ± 0.03$^{\#}$
Fontaine's stage	0.0 ± 0.0	1.8 ± 0.09$^{\#}$

Values are given as mean ± S.E.M. PAOD: peripheral arterial occlusive disease; BMI: body mass index; IHD: previous ischemic heart disease; sICAM-1: soluble intercellular adhesion molecule-1; sVCAM-1: soluble vascular cellular adhesion molecules-1; vWF: von Willebrand factor antigen; Hs-CRP: high sensitive C-reactive protein; BP: blood pressure; HDL: high-density lipoprotein cholesterol; HOMA: homeostatic model assessment index.

* $p < 0.05$ compared with Control subjects for each parameter.

$^{\#}$ $p < 0.01$ compared with Control subjects for each parameter.

3. Results

3.1. Study 1

The clinical and biochemical characteristics of the study subjects are shown in Table 1. We first examined the association between PAOD and adiponectin concentration. The concentration of adiponectin was significantly lower in PAOD subjects ($p < 0.01$). Furthermore, concentrations of sICAM-1 ($p < 0.03$) and sVCAM-1 ($p < 0.05$) were significantly higher in PAOD subjects. vWF and Hs-CRP levels tended to be higher in PAOD subjects than in Control subjects, but the differences were not significant. The uses of drugs that could influence adiponectin concentration such as angiotensin II receptor blockers or angiotensin-converting enzyme inhibitors (PAOD subjects; 45.0% versus Control subjects; 56.3%), and anti-diabetic agents (PAOD subjects; 15.0% versus Control subjects; 8.3%) were not significantly different between the two groups.

In the total subjects, adiponectin level was significantly correlated with BMI ($r = -0.22$, $p < 0.05$), triglycerides ($r = -0.25$, $p < 0.02$), HDL-cholesterol ($r = 0.45$, $p < 0.01$), creatinine ($r = 0.28$, $p < 0.01$), and Hs-CRP ($r = -0.26$, $p < 0.02$). Furthermore, adiponectin level was

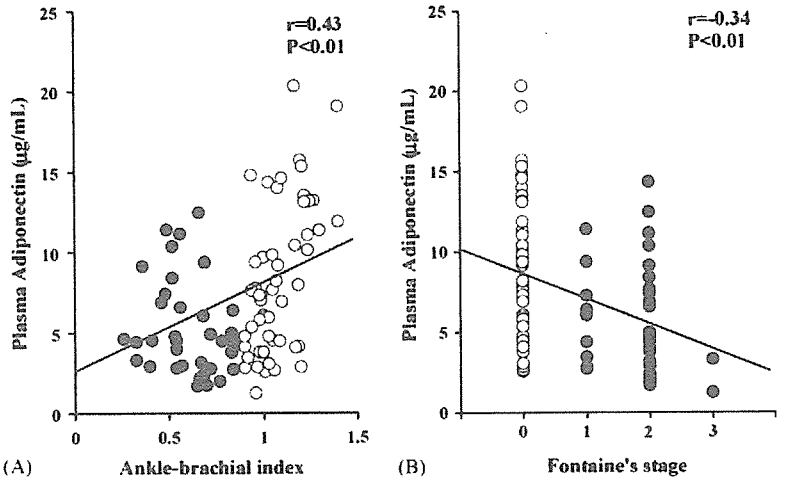

Fig. 1. Correlation of plasma adiponectin concentration with ankle–brachial index (A) and Fontaine's stage (B). Open circles indicate Control subjects, and closed circles indicate PAOD subjects.

significantly lower in male (6.0 ± 0.5 μg/mL versus 10.3 ± 0.9 μg/mL, $p < 0.01$), and diabetes (5.6 ± 0.7 μg/mL versus 7.9 ± 0.6 μg/mL, $p = 0.01$). On the other hand, there was no significant association between adiponectin and age ($r = 0.20$, NS), HOMA ($r = -0.13$, NS), and hypertension (6.6 ± 0.5 μg/mL versus 8.2 ± 1.0 μg/mL, NS). We next performed multiple regression analysis including age, sex, BMI, hypertension, diabetes, triglycerides, HDL-cholesterol, creatinine, sICAM-1, sVCAM-1, vWF, and Hs-CRP and reveled that adiponectin concentration in PAOD subjects was significantly lower than that in Control subjects (PAOD subjects; 7.9 ± 0.7 μg/mL versus Control subjects; 9.5 ± 0.6 μg/mL, $F = 4.94$, $p < 0.03$).

ABI and Fontaine's stage were significantly associated with adiponectin level (Fig. 1). Furthermore, ABI was also significantly associated with sICAM-1 and sVCAM-1 (Fig. 2), but not with vWF ($r = -0.11$, NS) and Hs-CRP ($r = -0.12$, NS). Multiple regression analysis including adiponectin, sICAM-1, sVCAM-1, vWF, and Hs-CRP was performed, and indicated that adiponectin ($F = 8.55$, $p < 0.01$) as well as sICAM-1 ($F = 5.8$, $p < 0.02$), sVCAM-1 ($F = 5.9$, $p < 0.02$), and Hs-CRP ($F = 3.8$, $p = 0.05$) were independently associated with ABI.

3.2. Study 2

As shown in Table 2, there were no significant differences between the two groups in clinical characteristics except for previous ischemic heart disease, ABI, and Fontaine's stage. The plasma concentration of adiponectin was significantly higher in the saphenous vein than in the femoral artery in subjects without PAOD (Table 3). That is, an approximately 13.0% step-up of adiponectin concentration from the femoral artery to the saphenous vein was found in subjects without PAOD. However, the step-up of adiponectin concentration from the femoral artery to the

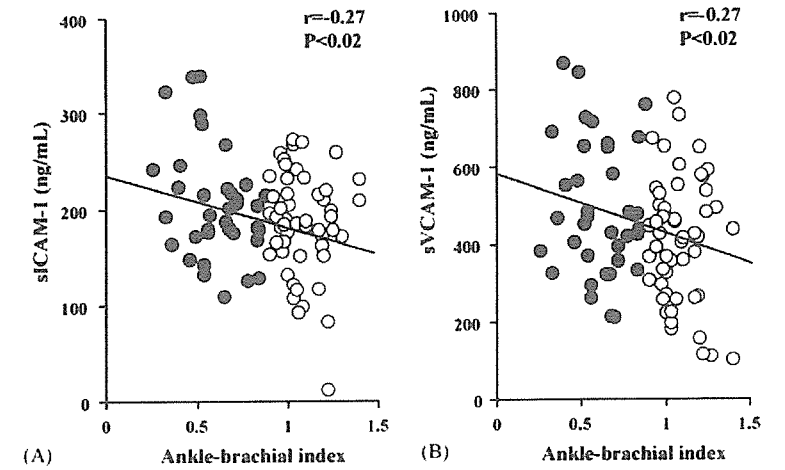

Fig. 2. Correlation of soluble intercellular adhesion molecule-1 (sICAM-1, A) and soluble vascular cellular adhesion molecules-1 (sVCAM-1, B) with ankle–brachial index. Open circles indicate Control subjects, and closed circles indicate PAOD subjects.

Table 2
Clinical variables of subjects in Study 2

Variables	Without PAOD, $n = 17$	PAOD, $n = 10$
Age (years)	64.7 ± 2.7	72.3 ± 2.0
Sex (male/female)	12/5	8/2
BMI (kg/m^2)	22.2 ± 0.8	21.9 ± 0.9
Smoking (%)	64.7	80.0
IHD (%)	64.71	100.0*
Diabetes (%)	35.3	60.0
Hypertension (%)	82.85	100.0
Hemoglobin (g/L)	129.82 ± 4.29	120.20 ± 4.52
Hematocrit	0.388 ± 0.011	0.363 ± 0.013
Systolic BP (mmHg)	133.8 ± 5.4	142.7 ± 7.8
Diastolic BP (mmHg)	74.5 ± 2.8	69.0 ± 4.5
Total cholesterol (mmol/L)	4.710 ± 0.196	4.482 ± 0.148
Triglycerides (mmol/L)	1.099 ± 0.134	1.240 ± 0.161
HDL-cholesterol (mmol/L)	1.219 ± 0.102	1.032 ± 0.089
HOMA	1.22 ± 0.17	1.73 ± 0.42
Serum creatinine (μmol/L)	89.46 ± 31.74	116.69 ± 25.37
Ankle–brachial index	1.14 ± 0.02	0.64 ± 0.06#
Fontaine's stage	0.0 ± 0.0	2.1 ± 0.3#

Values are given as mean ± S.E.M. PAOD: peripheral arterial occlusive disease; BMI: body mass index; IHD: previous ischemic heart disease; BP: blood pressure; HDL: high-density lipoprotein cholesterol; HOMA: homeostatic model assessment index.
* $p < 0.05$ compared with subjects without PAOD.
# $p < 0.01$ compared with subjects without PAOD.

Table 3
Plasma adiponectin concentration in femoral artery and saphenous vein

Parameters	Femoral artery	Saphenous vein	Δ(SV − FA)
PAOD, $n = 10$	5.924 ± 0.572	5.945 ± 0.521	0.021 ± 0.116
Without PAOD, $n = 17$	7.061 ± 1.244	7.95 ± 1.33#	0.891 ± 0.275¶

Data indicate mean ± S.E.M. SV: saphenous vein; FA: femoral artery; PAOD: Peripheral arterial occlusive disease.
# $p < 0.01$ vs. femoral artery of same group.
¶ $p < 0.01$ vs. PAOD.

saphenous vein disappeared in subjects with PAOD (+0.4%, NS).

3.3. Study 3

Changes in plasma concentrations of adiponectin and Hs-CRP in response to PTA are listed in Table 4. Hs-CRP concentration was significantly increased 2 days (+1936%) and 6 days (+700%) after PTA ($F = 18.8$, $p < 0.01$). Thus, PTA produced a significant increase in plasma levels of Hs-CRP, and their maximal increases were observed 2 days after PTA. On the other hand, adiponectin concentration showed a tendency to decrease after angioplasty (day 2, −20.1%; day 6, −30.6%, $F = 3.2$, $p = 0.07$).

Table 4
Plasma levels of adiponectin, and Hs-CRP before and after PTA

Parameters	Before	2 days	6 days
Adiponectin (μg/mL)	6.96 ± 2.14	5.56 ± 1.75	4.83 ± 1.50
Hs-CRP (mg/L)	1.1 ± 0.6	22.4 ± 4.1	8.8 ± 2.8

Values are given as mean ± S.E.M. PTA: percutaneous transluminal angioplasty; Hs-CRP: high sensitive C-reactive protein.

4. Discussion

The present study demonstrated that adiponectin concentration was decreased in subjects with PAOD, and the decrease was related to the clinical severity of this disease. Multiple regression analysis including age, sex, BMI, hypertension, diabetes, triglycerides, HDL-cholesterol, creatinine, sICAM-1, sVCAM-1, vWF, and Hs-CRP showed that adiponectin concentration was significantly lower in PAOD subjects. Furthermore, concentrations of adiponectin as well as sICAM-1, sVCAM-1, and Hs-CRP were independently associated with ABI. A significant step-up of adiponectin from the artery to the vein was observed in subjects without PAOD, but not in subjects with PAOD. In addition, adiponectin showed a tendency to decrease after PTA, although Hs-CRP increased.

Several studies have shown that plasma adiponectin concentration was decreased in various cardiovascular diseases [14,17]. However, little has been elucidated about the relation between plasma adiponectin and atherosclerotic lesions. Our present study showed that the plasma concentration of adiponectin in subjects with PAOD was significantly lower than that in those with similar atherosclerotic risk factors including hypertension, diabetes, and smoking but without PAOD. These risk factors were also known to affect plasma adiponectin concentration [14,15,21]. These results clearly indicate that atherosclerosis itself, apart from other influencing factors, associates the lower plasma adiponectin concentration in PAOD.

Atherosclerosis is a chronic process that involves cellular and humoral inflammatory response [2]. Previous studies had shown a reciprocal association of adiponectin with Hs-CRP and increased risk of arteriosclerosis [14,22]. These results suggest that a low adiponectin concentration might enhance the predisposition to PAOD via vascular inflammation. In the present study, however, plasma level of Hs-CRP, a marker of systemic inflammation, in PAOD subjects was not significantly higher than that in Control subjects. Although a previous study also showed no relationship between CRP, proinflammatory cytokines, and systemic atherosclerosis [23], there could be another mechanism that lower adiponectin leads to atherosclerosis. For example, adiponectin concentration was also shown to be independently correlated with the vasodilator response to reactive hyperemia, and adiponectin concentration could be an independent parameter of endothelial function [24], which is an important feature of the early stage of atherosclerosis. In addition, adiponectin may lower the risk of atherosclerosis by improving insulin sensitivity and blood lipid levels, as suggested by human data [25]. Furthermore, adiponectin suppresses lipid accumulation and class A scavenger receptor expression in macrophages and, consequently, the transformation of macrophages to foam