Counteractive effects of omentin-1 against atherogenesis†

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Aims
Omentin-1, a novel adipocytokine expressed in visceral fat tissue, is negatively correlated with obesity, insulin resistance, and stable coronary artery disease (CAD). However, there have been no previous reports regarding the effects of omentin-1 on atherogenesis.

Methods and results
This study was performed to evaluate the atheroprotective effects of omentin-1 on human monocyte-derived macrophages, human aortic smooth muscle cells (HASMCs) in vitro, and aortic lesions in Apoe−/− mice in vivo. The histological expression of omentin-1 in coronary artery lesions and epicardial adipose tissues and its plasma levels were compared between CAD and non-CAD patients. Omentin-1 was abundantly expressed in human umbilical vein endothelial cells, macrophages, HASMCs, and human coronary artery SMCs in vitro. Omentin-1 promoted anti-inflammatory M2 phenotype during differentiation of human monocytes into macrophages. Omentin-1 suppressed oxidized low-density lipoprotein-induced foam cell formation associated with down-regulation of CD36, scavenger receptor class A, and acyl-CoA:cholesterol acyltransferase-1 and up-regulation of neutral cholesterol ester hydrolyase in human macrophages. Omentin-1 suppressed angiotensin II-induced migration and platelet-derived growth factor-BB-induced proliferation, and collagen-1 and -3 expression in HASMCs. Four-week infusion of omentin-1 into Apoe−/− mice retarded the development of aortic atherosclerotic lesions with reduced contents of monocytes/macrophages, SMCs, and collagen fibres along with peritoneal M2-activated macrophages with inflammasome down-regulation and lowered plasma total cholesterol levels. Omentin-1 levels were markedly reduced in coronary endothelium and epicardial fat but increased in plasma and atheromatous plaques (macrophages/SMCs) in CAD patients compared with non-CAD patients.

Conclusion
This study provided the first evidence that omentin-1 may serve as a novel therapeutic target for atherosclerosis and CAD.

1. Introduction
Metabolic syndrome with visceral fat accumulation is a major risk factor for atherosclerotic cardiovascular disease.1 Adipose tissue produces a number of adipocytokines, such as leptin and adiponectin, which play crucial roles in modulating atherosclerosis.1 Atherosclerosis is a pathological injury-to-response process that is initiated by early inflammatory responses with up-regulations of intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), and cyclooxygenase-2 (COX-2) in endothelial cells (ECs) and monocyte adhesion and infiltration into neointima lesions, followed by fatty streak formation with subendothelial accumulation of lipid-laden macrophage foam cells.2 Foam cell formation is characterized by cholesterol ester (CE) accumulation that depends on the balance between the uptake of oxidized low-density lipoprotein (oxLDL) via scavenger receptors, such as CD36, scavenger receptor class A (SR-A), and lectin-like

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oxidized low-density lipoprotein receptor-1 (LOX-1), and the efflux of free cholesterol (FC) controlled by ATP-binding cassette transporter A1 (ABCA1). Intracellular CE accumulation is also regulated by the balance between cholesterol esterification by acyl-CoA:cholesterol acyltransferase-1 (ACAT-1) and hydrolysis by neutral cholesteryl ester hydrolase (NCEH). Macrophage phenotype characterized as pro-inflammatory (M1) or anti-inflammatory (M2) has been recently focused on foam cell formation and atherosclerosis. In addition, vascular smooth muscle cells (VSMCs) contribute to progression of atherosclerotic plaque by their migration, proliferation, and production of extracellular matrix (ECM) components, such as collagen, matrix metalloproteinases (MMPs), fibronectin, and elastin.

Omentin-1, also named intelectin-1, is a newly identified adipocytokine of 313 amino acids originally identified in an omental fat cDNA library. Human omentin-1 and mouse omentin-1 are >84.9% identical. The receptors for omentin-1 are still not elucidated. Several lines of experimental and clinical evidence have shown that omentin-1 stimulates insulin-mediated glucose uptake in human adipocytes, and is negatively correlated with obesity, insulin resistance, diabetes, metabolic syndrome, and stable coronary artery disease (CAD).

Omentin-1 induces vasodilation in rat isolated blood vessels by increasing endothelial nitric oxide synthase, stimulates ischaemia-induced revascularization, and prevents myocardial ischaemic injury- and arterial injury-induced neointimal formation in mice. Omentin-1 inhibits tumour necrosis factor-α (TNF-α)-induced expression of ICAM-1, VCAM-1, and COX-2 in human umbilical vein endothelial cells (HUVECs) and adhesion of monocytes to HUVECs. In addition, omentin-1 has also been shown to inhibit TNF-α-induced VCAM-1 expression, platelet-derived growth factor-BB (PDGF-BB)-induced migration, superoxide production, and calcification in VSMCs. However, there have been no previous reports regarding the effects of omentin-1 on atherosclerosis.

In the present study, we evaluated the suppressive effects of omentin-1 on inflammatory response and foam cell formation in macrophages, VSMC migration and proliferation, and ECM production by VSMCs in vitro, and the development of aortic atherosclerotic lesions in ApoE−/− mice in vivo. Further, the levels of omentin-1 expression in the circulating blood, coronary atherosclerotic lesions, and epicardial adipose tissues were compared between CAD and non-CAD patients.

2. Methods

2.1 Human blood and coronary atherosclerotic lesion samples

This study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethics Committee of Showa University and Tokyo University of Pharmacy and Life Sciences. Informed consent was obtained from all participants prior to enrolment. Blood was collected from 69 male patients with acute coronary syndrome (ACS) hospitalized at Showa University Hospital for emergent coronary catheterization (aged 40–96), 21 male hypertensive patients (aged 36–78) as non-CAD patients, and 21 male healthy volunteers (aged 22–59). The prevalence rate of obesity, diabetes, hypertension, or dyslipidemia and the main medications are listed in Table 1. Plasma omentin-1 concentration was measured by enzyme-linked immunosorbent assay (human omentin-1 ELISA kit, Aviscera Bioscience, Santa Clara, CA, USA).

Paraffin-embedded tissue archive collections of the National Cerebral and Cardiovascular Center from human coronary arteries, epicardial adipose tissues, and the greater omentum obtained from seven male patients with acute and prior myocardial infarction (aged 62–87) and five male patients with dilated cardiomyopathy (aged 19–46) as non-CAD patients at autopsy were used for immunohistochemistry. Paraffin blocks of the coronary arteries with epicardial fat from CAD and non-CAD patients were used after fixation in buffered 10% formalin solution. The 3–4 µm sections were serially cut and stained with haematoxylin–eosin and Elastica Van Gieson. Immunohistochemistry was performed on deparaffinized glass slides by the automated immunostainer BOND-III-TM (Leica Biosystems, Newcastle, UK). We used a mouse monoclonal antibodies against human macrophages (CD68; DAKO, Tokyo, Japan) or α-smooth muscle actin (α-SMA; DAKO) and rabbit polyclonal antibodies against human von Willebrand factor (vWF; DAKO) or human omentin-1 (LifeSpan BioSciences, Seattle, WA, USA) as a primary antibody. The detection kit as a secondary antibody was the Bond Polymer Refine Detection (DS9800, Leica Biosystems); it involved incubation with post primary for 8 min, polymer for 8 min, diaminobenzidine for 10 min, and haematoxylin for 5 min. Masson’s Trichrome staining for collagen fibres was also performed.

2.2 Human monocyte primary culture

This investigation was approved by the Ethics Committee of Tokyo University of Pharmacy and Life Sciences. Informed consent was obtained from all participants. Human peripheral mononuclear cells were isolated from the blood of 24 healthy volunteers (aged 20–24). Monocytes purified using anti-CD14 antibody-conjugated magnetic microbeads (Miltenyi Biotec, Auburn, CA, USA) were seeded onto 3.5 cm dishes (1 × 10⁶ cells/mL) for 7 days in RPMI-1640 medium supplemented with 10% human serum, 0.05 mM streptomycin, 50 U/mL penicillin, and the indicated concentrations of human omentin-1 (Aviscera Bioscience). The medium in each dish was replaced with fresh medium containing omentin-1 every 3.5 days.

2.3 Cholesterol esterification assay

Human macrophages differentiated by 7-day culture as described above were incubated for 19 h with the same concentrations of omentin-1 along with 50 µg/mL human oxLDL and 0.1 mmol/L [3H]oleate (PerkinElmer, Yokohama, Japan) conjugated with bovine serum albumin. Cellular lipids were extracted and the radioactivity of cholesterol-[3H]oleate was determined by thin-layer chromatography.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics</th>
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<tr>
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<td>100</td>
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<tr>
<td>Age (years)</td>
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<td>Dyslipidemia</td>
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<td>Anti-diabetic drugs (%)</td>
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<tr>
<td>ARBs (%)</td>
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<tr>
<td>Statins (%)</td>
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</tbody>
</table>

Values are expressed as mean ± SEM or percentage. *P < 0.0001 vs. healthy volunteers; †P < 0.01 vs. non-CAD patients; ‡P < 0.01, ‰P < 0.001 vs. CAD patients.
2.4 Migration assay

Human aortic smooth muscle cells (HASMCs; Lonza, Walkersville, MD, USA) at passage 7 or 8 were seeded onto 8-well culture slide (3 × 10^4 cells/200 µL/well). Cells were incubated at 37°C in 5% CO₂ for 3–5 h in smooth muscle cell basal medium (SmBM; Lonza) supplemented with 0.5 nmol/L human epidermal growth factor, 5 µg/mL insulin, 2 ng/mL human fibroblast growth factor, 50 µg/mL gentamicin, 50 ng/mL amphotericin B, and 5% FBS, and were then incubated for 24 h in serum-free SmBM. Subsequently, while cells were incubated for 15 h in serum-free SmBM with the indicated concentrations of angiostatin II (AngII; Sigma, St. Louis, MO, USA) and/or omentin-1, photographs of cells were taken for the last 5 h at 10 min intervals. The average migration distance of 10 cells randomly selected in each well was measured using a BIOREVO BZ-9000 microscope (Keyence, Osaka, Japan).

2.5 Proliferation assay

HASMCs at passage 7 or 8 were seeded onto 96-well plates (1 × 10⁴ cells/100 µL/well) and incubated for 24 h in the same 5% FBS–SmBM supplemented with the indicated concentrations of PDGF-BB (Wako, Osaka, Japan). HASMCs were further incubated for 48 h with the indicated concentrations of PDGF-BB and/or omentin-1 with renewal of each medium. Then, 10 µL of WST-8 solution (Cell Count Reagent SF; Nacalai Tesque, Kyoto, Japan) was added to each well. After 1 h of incubation, the amount of formazan product was determined by measuring the absorbance at 450 nm using a Sunrise Remote RTM-microplate reader (Tecan, Kawasaki, Japan).

2.6 Immunoblotting analysis

Equal amounts of protein lysates from human macrophages and HASMCs were separated by 10% SDS–PAGE and subjected to immunoblotting with specific antibodies to human omentin-1, MRC1 (LifeSpan BioSciences), phosphoinositide 3-kinase (PI3K), Raf-1, nuclear factor-kB (NF-kB), NCEH, LOX-1 (Abcam, Tokyo, Japan), ACAT-1, CD68 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), peroxisome proliferator-activated receptor-α (PPAR-α; Signalway Antibody, College Park, MD, USA), CD36, SR-A (R&D Systems, Minneapolis, MN, USA), ABCA1, α-tubulin, collagen-1 (Novus Biologicals, Littleton, CO, USA), collagen-3, fibronectin, MMP-2 (GeneTex, Irvine, CA, USA), and adipose tissues were carefully removed. The entire aorta and cross sections of the aortic sinus were stained with oil red O for assessment of atherosclerotic lesions. The immunohistochemical expression of omentin-1 was detected by anti-human omentin-1 antibody (LifeSpan BioSciences). Monocytes/macrophages and VSMCs in the aortic atherosclerotic lesions were visualized by immunostaining with antibodies against MOMA-2 (Millipore, Billerica, MA, USA) or α-SMA (Sigma), respectively. Collagen fibres were stained blue with Masson’s Trichrome. These areas of the aortic wall were traced by an investigator blind to the treatment and measured using image analysis software (Adobe Photoshop, San Jose, CA; NIH ImageJ, Bethesda, MD, USA).

2.7 Animal experiments

Animal experiments were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Tokyo University of Pharmacy and Life Sciences. A total of 46 male spontaneously hyperlipidaemic Apo⁻/⁻ mice (C57BL/6.KOR/StmSic-Apo⁻/⁻ mice) at the age of 9 weeks were purchased from Japan SLC Inc. (Hamamatsu, Japan) and maintained on a normal diet until the age of 13 weeks. Then, a high-cholesterol diet (Oriental Yeast, Tokyo, Japan) and/or omentin-1 antibodies to human omentin-1 (Abcam) at doses of 0, 2.5, and 5 µg/kg for 4 weeks using osmotic mini-pumps (Alzet Model 1002; Durect, Cupertino, CA, USA).

2.8 Mouse atherosclerotic lesion assessment

Two groups were involved. The body weight and blood pressure were measured before and after 4-week infusion. Food intake was measured daily for 2 weeks prior to the experimental endpoint. Systolic and diastolic blood pressures were measured using the indirect tail-cuff method (Kent Scientific, Torrington, CT, USA). Four days before the endpoint of infusion, mice were ip injected with 2 mL of aged-autochthonated thiglycolate broth to obtain exudate peritoneal macrophages, which is thought to mimic arterial macrophages present in atheroma areas. Exudate peritoneal macrophages are widely accepted to use in the assessment of mouse atherosclerosis.

At the experimental endpoint, exudate peritoneal cells were sampled by needle aspiration from the peritoneal cavity and cell viability was assessed with trypan blue. Then, cells were resuspended in HBSS and counted using a FACSCalibur (Becton Dickinson, San Jose, CA) and BD CellQuest software (Becton Dickinson, San Jose, CA). The cell viability was determined by measuring the absorbance at 450 nm using a Sunrise Remote RTM-microplate reader (Tecan, Kawasaki, Japan).

2.9 Mouse exudate peritoneal macrophage assessment

Exudate peritoneal cells obtained immediately after 1 week of infusion were seeded onto 96-well plates (1 × 10⁴ cells/200 µL/well) and incubated for 24 h in the same 5% FBS–SmBM supplemented with the indicated concentrations of PDGF-BB and/or omentin-1 with renewal of each medium. Then, 10 µL of WST-8 solution (Cell Count Reagent SF; Nacalai Tesque, Kyoto, Japan) was added to each well. After 1 h of incubation, the amount of formazan product was determined by measuring the absorbance at 450 nm using a Sunrise Remote RTM-microplate reader (Tecan, Kawasaki, Japan).

2.10 Mouse blood sample measurement

Blood samples collected in tubes with heparin were centrifuged at 1000 g for 10 min. Fasting plasma glucose, total cholesterol, and high-density lipoprotein (HDL) cholesterol concentrations were measured by enzymatic methods (Denka Seiken, Tokyo, Japan) and a precipitation method (Wako). Plasma omentin-1 concentration was measured using the ELISA kit (Aviscera Bioscience).
3. Results

3.1 Expression of omentin-1 in human normal vascular cells in vitro

Expression of omentin-1 was observed at appreciable levels in vascular cells in addition to 3T3-L1 adipocytes that showed high levels of expression (Figure 1A). Listed in order of expression level, omentin-1 was expressed in HUVECs/human macrophages, HASMCs/human coronary artery smooth muscle cells (HCASMC; Lonza), and human monocytes (Figure 1A).

3.2 Effects of omentin-1 on human VSMC migration and proliferation

Our results and those of another group reported previously indicated that omentin-1 itself had no significant effects on migration or proliferation of VSMCs.20 Therefore, we assessed the effects of omentin-1 on AngII-induced migration and PDGF-BB-induced proliferation of HASMCs. AngII (500 nmol/L) significantly increased the migration of HASMCs (P < 0.0001; Figure 1B). Omentin-1 (200 ng/mL) significantly suppressed the AngII-induced migration of HASMCs (P < 0.0001; Figure 1B). PDGF-BB (10 ng/mL) significantly increased the proliferation of HASMCs (P < 0.01; Figure 1C). Omentin-1 (200 ng/mL) significantly suppressed the PDGF-BB-induced proliferation of HASMCs (P < 0.05; Figure 1C). Omentin-1 (200 ng/mL) time-dependently suppressed both contractile and synthetic phenotypes (α-SMA and SMemb) with downregulation of PI3K, c-Src, Raf-1, ERK-1/2, and NF-κB, but not AKT and α-tubulin, in HASMCs (Figure 1D).

3.3 Effects of omentin-1 on phenotype in human monocyte/macrophage differentiation

After 3–6 days of primary culture, the differentiation of human monocytes into macrophages was confirmed by increased expression of CD68, a macrophage differentiation marker (Figure 1E). Omentin-1 did not affect monocytic differentiation into macrophages and ERK-1/2...
and β-actin expression. However, omentin-1 decreased the expression of MARCO, an M1 marker, and NF-κB, but increased that of MRC1, an M2 marker, and PPAR-γ (Figure 1E). These observations suggested that omentin-1 shifted the macrophage phenotype overwhelmingly to M2 rather than M1 via PPAR-γ up-regulation and NF-κB down-regulation.

3.4 Effects of omentin-1 on human macrophage foam cell formation

Omentin-1 significantly suppressed oxLDL-induced foam cell formation by 20% at 10 ng/mL in human monocyte-derived macrophages (P < 0.05; Figure 1E). Omentin-1 at 500 ng/mL significantly suppressed CD36, SR-A, and ACAT-1 protein expression by 80, 60, and 40%, respectively (all P < 0.05; Figure 2). However, omentin-1 significantly increased LOX-1 and NCEH protein expression by 2.6-fold and 2.2-fold, respectively (both P < 0.05; Figure 2). Omentin-1 showed no significant effects on ABCA1 protein expression (P = NS; Figure 2).

3.5 Effects of omentin-1 on ECM expression in human VSMCs

Omentin-1 significantly suppressed collagen-1 and collagen-3 protein expression, but increased that of elastin and fibronectin in HASMCs (P < 0.01, P < 0.05; Figure 3). However, omentin-1 had no significant effect on MMP-2 or MMP-9 expression in HASMCs (both P = NS; Figure 3).

3.6 Effects of omentin-1 on atherosclerotic lesion development in Apoe-/- mice

In Apoe-/- mice, aortic atherosclerotic lesions increased markedly in size by three-fold as well as body weight at 21 weeks of age compared with 17 weeks of age (Figure 4A, B, E, F, U, and V, Table 2). Among the three groups at 21 weeks of age, plasma levels of omentin-1 did not differ significantly contrary to expectations (Table 2), but the tissue and cellular expression levels of omentin-1 in the aortic root adventitia, its surrounding myocardium, and exudate peritoneal macrophages were visibly elevated by infusion of omentin-1 (Figure 5A and C). The omentin-1-positive areas in the aortic wall were significantly greater in mice infused with 2.5 and 5 μg/kg/h of omentin-1 compared with counterparts (Figure 5B).

Chronic infusion of omentin-1 at 2.5 and 5 μg/kg/h significantly reduced the surface areas of the atherosclerotic lesions with obvious reductions in monocyte/macrophage infiltration, VSMC contents, and collagen fibres within atheromatous plaques in the aortic root.

![Figure 2](https://academic.oup.com/cardiovascres/article-abstract/110/1/118/2463190) Effects of omentin-1 on foam cell formation related protein expression in human monocyte-derived macrophages. Human monocytes were incubated for 7 days with omentin-1 (0–500 ng/mL), and the cells were then harvested for immunoblotting analyses of CD36, SR-A, LOX-1, ACAT-1, NCEH, ABCA1, or β-actin. (Top) Representative results of protein expression of each molecule. (Bottom) Densitometric data of each molecule after normalization relative to β-actin. Data are expressed as means ± SEM from six to eight independent experiments with monocytes from six to eight different donors. *P < 0.05 vs. 0 ng/mL of omentin-1.
Either 2.5 or 5 mg/kg/h or both of omentin-1 significantly reduced the number of exudate peritoneal cells (P, 0.0005; Table 2) and suppressed protein expression of MARCO, an M1 marker, ASC, NF-κB, C-reactive protein, COX-2, PI3K, c-Src, Raf-1, and ERK-1/2, but promoted that of arginase-1, a mouse M2 marker, and PPAR-γ in macrophages (Figure 5C). Omentin-1 did not affect AKT and β-actin expression.

As shown in Table 2, there were no significant differences in body weight, food intake, systolic and diastolic blood pressures, or plasma HDL cholesterol concentration among the three groups of 21-week-old Apoe<sup>2</sup>/2 mice. However, plasma glucose concentration was somewhat decreased in only Apoe<sup>2</sup>/2 mice infused with a high dose of omentin-1 (5 mg/kg/h; P = NS). Both doses of omentin-1 significantly lowered plasma total cholesterol concentration (both P, 0.0001).

### 3.7 Expression of omentin-1 in human omentum, epicardial fat, and coronary artery lesions

The immunohistochemical expression of omentin-1 was weaker in the omentum and markedly reduced in epicardial adipose tissues in CAD patients compared with non-CAD patients (Figure 6A–D). Omentin-1 was intensely positive in endothelium of coronary arteries in non-CAD patients (Figure 6F). In contrast, omentin-1 was weakly positive in endothelium (Figure 6G) where it was positive by vWF (Figure 6H), but intensely positive in atheromatous plaques of CAD patients (Figure 6G). Within advanced atherosclerotic plaques of coronary arteries, omentin-1 was positive in monocyte-derived macrophages (foam cells) and medial layer VSMCs, as indicated by consistent areas stained with anti-CD68 and anti-α-SMA antibodies (Figure 6I–K).

### 3.8 Plasma concentration of omentin-1 in ACS patients

As shown in Table 1, male ratio, body weight, and body mass index (BMI) did not differ significantly among ACS (CAD) patients, hypertensive (non-CAD) patients, and healthy volunteers. Although there was a significant difference in age among the three groups, Pearson’s regression analysis revealed no significant correlation between age and plasma omentin-1 concentration in all the subjects (r = 0.062, P = NS). The ratios of hypertension and AngII receptor blockers (ARBs) use were significantly greater in non-CAD patients compared with others (Table 2). The prevalence of obesity, diabetes, dyslipidemia, and use of anti-diabetic drugs and statins did not differ significantly between non-CAD and CAD patients (Table 2). Additionally, there were no significant differences in haemoglobin A1c (5.5 ± 0.3 vs. 5.8 ± 0.2%, P = NS) and total cholesterol levels (198.8 ± 7.3 vs. 183.2 ± 5.0 mg/dL, P = NS) between non-CAD and CAD patients. As shown in Figure 6M, plasma omentin-1 concentrations were significantly elevated in CAD patients than non-CAD patients and healthy volunteers (P < 0.005, P < 0.05). Plasma omentin-1 concentration was remarkably
but not significantly lower in non-CAD patients than healthy volunteers ($P = \text{NS}$).

4. Discussion

This is the first demonstration that omentin-1 promotes macrophage differentiation into the anti-inflammatory M2 phenotype, and suppresses the inflammatory responses and oxLDL-induced foam cell formation in macrophages. AngII-induced migration and PDGF-BB-induced proliferation of VSMCs, and collagen-1 and -3 expression in VSMCs in vitro, and retards the development of atherosclerotic lesions with reduced monocyte/macrophage infiltration, VSMC contents, and collagen fibres in ApoE$^{-/-}$ mice in vivo. Omentin-1 expression is decreased in epicardial adipose tissues and coronary endothelium in patients with CAD. However, omentin-1 is markedly increased in macrophage-derived foam cells and medial layer VSMCs within advanced coronary plaques and the circulating blood in patients with ACS. These findings suggest that omentin-1 may counteract the progression of atherosclerosis. Our translational research combining with human cellular, animal, and clinical experiments is essential to elucidate comprehensively the roles of omentin-1 in atherosclerosis.

Omentin-1 is a novel soluble lectin that is preferentially produced by visceral adipose tissue compared with subcutaneous adipose tissue, and is also expressed in epicardial fat and ECs. Omentin-1 is negatively associated with endothelial dysfunction and arterial stiffness and calcification. The results of the present study indicated that omentin-1 increases the expression of elastin and fibronectin in VSMCs, contributing to arterial elasticity. Omentin-1 is inversely associated with the severity of carotid atherosclerotic lesions in patients with metabolic syndrome or hypertension. In our study, plasma omentin-1 levels were reduced in hypertensive non-CAD patients, because they had obesity, diabetes, and dyslipidemia. Omentin-1 is negatively correlated with BMI, insulin resistance, total cholesterol, and haemoglobin A1c and positively correlated with adiponectin and HDL cholesterol concentrations and very low-density lipoprotein (VLDL) size. Circulating omentin-1 level increases after weight loss, aerobic training, and treatment with metformin or the glucagon-like peptide-1 analogue, exanetide, via improved insulin sensitivity.

Figure 4  Effects of omentin-1 on the development of atherosclerotic lesions in ApoE$^{-/-}$ mice. Ten mice sacrificed before infusion (17 weeks old), and 15, 10, and 11 mice after 4-week infusion of human omentin-1 at doses of 0, 2.5, and 5 $\mu$g/kg/h, respectively, were used. Atherosclerotic lesions were stained with oil red O on the aortic surface (A–D). Scale bar = 5 mm. Cross sections of the aortic root were stained with oil red O (E–H), MOMA-2 for monocytes/macrophages (I–L), $\alpha$-SMA for VSMCs (M–P), or Masson’s Trichrome for collagen fibres (Q–T). Haematoxylin was used for nuclear staining. Scale bar = 500 $\mu$m. Data are expressed as means $\pm$ SEM. *$P < 0.0001$, †$P < 0.01$, ‡$P < 0.05$ vs. before infusion of omentin-1 (17 weeks old); $\dagger$P < 0.0001, $\ddagger$P < 0.001, $\mathsection$P < 0.05 vs. 0 $\mu$g/kg/h of omentin-1 (21 weeks old).
### Table 2  Characteristics and laboratory data in Apoe<sup>−/−</sup> mice

<table>
<thead>
<tr>
<th></th>
<th>Before infusion</th>
<th>Omentin-1 0 µg/kg/h</th>
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<th>Omentin-1 5 µg/kg/h</th>
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<tr>
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<td>21 ± 0</td>
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<td><strong>Body weight (g)</strong></td>
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<td>30.3 ± 0.4*</td>
<td>29.7 ± 0.4</td>
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<td><strong>Food intake (mg)</strong></td>
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<td><strong>Systolic blood pressure (mmHg)</strong></td>
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<td><strong>Diastolic blood pressure (mmHg)</strong></td>
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<td>82.5 ± 2.9</td>
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<td><strong>Omentin-1 (ng/mL)</strong></td>
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<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
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<td><strong>Glucose (mg/dL)</strong></td>
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<td><strong>Total cholesterol (mg/dL)</strong></td>
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<td>1764.4 ± 60.1</td>
<td>1129.4 ± 45.7†</td>
<td>1339.1 ± 60.0†</td>
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<td><strong>HDL cholesterol (mg/dL)</strong></td>
<td>6.7 ± 1.5</td>
<td>6.6 ± 1.1</td>
<td>4.3 ± 0.8</td>
<td>4.7 ± 1.0</td>
</tr>
<tr>
<td><strong>Exudate peritoneal cells (×10^7) NE</strong></td>
<td>0.60 ± 0.07</td>
<td>0.24 ± 0.06↑</td>
<td>0.25 ± 0.04↑</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM or percentage.

NE, not examined.

*P < 0.05 vs. before infusion of omentin-1 (17 weeks old); †P < 0.0001; ‡P < 0.0005 vs. 0 µg/kg/h of omentin-1.

### Figure 5  Expression of omentin-1 in aortic adventitia, myocardium, and peritoneal macrophages in Apoe<sup>−/−</sup> mice.

(A) Oil red O staining (upper panels) and immunohistochemical expression of omentin-1 (lower panels) in the aorta and myocardium among Apoe<sup>−/−</sup> mice infused with different doses of human omentin-1 (0, 2.5, and 5 µg/kg/h) are shown. Omentin-1 is intensively positive in adventitia and the surrounding myocardium. AoV, aortic valve; Adv, adventitia; P, plaque; M, myocardium. Scale bars = 100 µm. (B) The omentin-1-positive areas in the aortic wall are greater in omentin-1-infused mice than counterparts. *P < 0.0001, †P < 0.005. (C) Immunoblotting of phenotypes and inflammasomes in exudate macrophages from Apoe<sup>−/−</sup> mice infused with human omentin-1 was analyzed by 10% SDS–PAGE. Immunoblotting analyses of human omentin-1, MARCO, arginase-1, C-reactive protein (CRP), ASC, NF-κB, COX-2, ERK-1/2, PPAR-γ, AKT, and β-actin were independently repeated at least three times.
Epicardial adipose tissues are associated with atherosclerotic lesions in the coronary arteries. Increased adipocyte size, increased levels of inflammatory cytokines, and decreased levels of adiponectin have been reported in patients with CAD. Our study indicated that expression levels of omentin-1 as well as adiponectin (data not shown) are reduced in the epicardial adipose tissues of patients with CAD. Similar to adiponectin, omentin-1 could reach into the coronary artery wall via paracrine and vasocrine secretion from epicardial adipose tissues other than hormonal circulation. However, the reduced production of omentin-1 in epicardial and visceral fat tissues and coronary artery ECs leads to atherosclerotic plaque formation. Therefore, it is possible that omentin-1 expression in macrophages and VSMCs within advanced plaques may increase to react against plaque progression. Serum levels of omentin-1 are transiently increased with the rupture of coronary plaques at the onset of ACS. On the basis of these findings, we speculate that omentin-1 may be not only a negative risk factor for chronic CAD but also a positive acute-phase reactant in ACS for the sake of cardiovascular protection.

We discuss the integrity of omentin-1 concentrations in our in vitro and in vivo experiments. First, the concentrations of omentin-1 required for modulation of several responses of human macrophages and HASMCs were relatively high (2.3- to 113-fold) compared with plasma omentin-1

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**Figure 6** Expression of omentin-1 in human coronary arteriosclerotic lesions, epicardial adipose tissues, and omentum, and its plasma concentration. Representative cases with non-CAD (A, C, and F) and CAD (B, D, and G–L) show immunohistochemistry of omentin-1 on their omentum (A and B), epicardial adipose tissues (C and D), and coronary arteries (E–G and I), vWF (EC marker; H), CD68 (macrophage marker; J), and α-SMA (VSMC marker; K), and Masson’s Trichrome (blue area shows collagen fibres; L). Panels A and C show more intense omentin-1 in omental and epicardial fat of non-CAD than those of CAD (B and D). Panel F shows omentin-1 in coronary endothelium of non-CAD case compared with endothelium of CAD (G). Panel I shows omentin-1 positive in area of CD68 positive (J) and α-SMA-positive medial area (K). Panel M shows the increased plasma omentin-1 concentration in 69 CAD patients compared with 21 non-CAD patients and 21 healthy volunteers. *P < 0.05, †P < 0.005.
concentrations in humans (4.4 ng/mL). The visceral fat is the main source of circulating omentin-1. Further paracrine and vasocore secretion of omentin-1 occurs from epididymal fat and coronary arterial cells. Therefore, it is not surprising that local levels of omentin-1 were increased similar to other vasocore peptides, such as AngI.46 Secondly, the concentration of omentin-1 in human serum from a healthy volunteer was 1.1 ng/mL in the present cell culture experiments. Therefore, the 10% concentration added to the culture medium for monocytes—macrophages (0.11 ng/mL) was negligible compared with the concentrations of omentin-1 added. Thirdly, the adequate concentrations of omentin-1 differed in inducing VSMC proliferation, ECM production, macrophage foam cell formation, and related protein expression. This is mostly dependent on the difference in cell types and their intracellular signalling pathways. Last, increased plasma concentrations of omentin-1 were not observed in Apoe−/− mice infused with human omentin-1 against our expectations. Instead, we demonstrated the plasma biomarkers, are expected to emerge as a new line of therapies against CAD. Thus, the 10% concentration added in vivo may decrease intestinal cholesterol absorption and hepatic VLDL synthesis and assembly, and/or may increase hepatic LDL receptor expression, leading to a decrease in plasma concentration of total cholesterol in vivo. Among human macrophage scavenger receptors, only LOX-1 was expressed at high levels in vitro. LOX-1 may be up-regulated for compensatory uptake of oxLDL to avoid intracellular FC detection as a result of CD36 and SR-A down-regulation in human macrophages. In the same way as adiponectin, omentin-1 shifts human monocytes into alternative anti-inflammatory M2 macrophages associated with PPAR-γ up-regulation and NF-κB down-regulation.47,48

In conclusion, the present study provided the first evidence that omentin-1 exerts anti-atherogenic effects by lowering plasma total cholesterol levels and by suppressing inflammatory response and foam cell formation in macrophages, and the migration, proliferation, and collagen production by VSMCs. This may open up a new therapeutic window for combating atherosclerosis and related diseases. Thus, omentin-1-based treatments, with this peptide itself and/or its analogues, are expected to emerge as a new line of therapies against CAD.

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