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ORIGINAL ARTICLE

Association of a fat-derived plasma protein omentin with carotid artery intima-media thickness in apparently healthy men

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Obesity is causally linked with the development of atherosclerosis. Omentin is an adipocytokine whose concentrations are reduced in obese individuals. Here we examined the relationship between plasma omentin levels and carotid intima-media thickness (IMT), a marker of early atherosclerosis, in apparently healthy Japanese men. Participants were 100 Japanese men who underwent a medical checkup. Maximal IMT (max-IMT) and mean-IMT in common carotid artery were measured by high-resolution carotid ultrasound system. Plasma omentin concentrations were determined by enzyme-linked immunosorbent assay. Circulating omentin levels correlated negatively with body mass index, waist circumference, fasting glucose, creatinine, max-IMT and mean-IMT, and positively with estimated glomerular filtration rates (eGFR). Single regression analysis demonstrated that max-IMT associated with age, eGFR and omentin levels, and that mean-IMT associated with age, fasting glucose, eGFR and omentin levels. Multiple regression analysis revealed that omentin levels, together with age, correlated with max-IMT and mean-IMT. Our data document that circulating omentin levels independently and negatively associate with carotid IMT in this population, suggesting that measurement of omentin may be useful for assessment of carotid IMT.
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INTRODUCTION

Obesity, in particular, excess visceral fat depot, is highly associated with dyslipidemia, type 2 diabetes and hypertension, finally resulting in atherosclerotic cardiovascular disease.[1] A growing of evidence indicates that adipose tissue affects a number of remote organs including the vasculature by producing various bioactive substances, also referred to as adipocytokines or adipokines.[1–3] It has been suggested that the imbalance in the production of different adipocytokines contributes to the development of obesity-linked complications.[1–3]

Omentin, also known as intelectin-1, is a recently identified adipocytokine, whose expression is abundantly detected in human visceral fat tissue.[4–6] Omentin is detectable in human plasma and is downregulated in subjects with obesity and type 2 diabetes.[7,8] Low levels of plasma omentin are also associated with insulin resistance and endothelial dysfunction.[9,10] Thus, it is conceivable that circulating omentin is associated with obesity-linked disorders. An increase in carotid arterial intima-media thickness (IMT) is an established marker of early atherosclerosis and is predictive of future cardiovascular and stroke events.[11,12] In the present study, we investigated the association between circulating omentin levels and carotid IMT in apparently healthy Japanese males.

METHODS

Study population

Participants in this study were apparently healthy subjects who visited Chunichi Hospital in Nagoya for a routine checkup between 2006 and 2009. A total of 100 Japanese men with no history of cardiovascular disease, who were not taking any medication, participated in this study. All subjects enrolled in this study provided written informed consent. This study was approved by the Ethics Committee of Nagoya University School of Medicine and Chunichi Hospital.

Measurement of carotid IMT

IMT in common carotid artery was measured by high-resolution carotid ultrasound system using the Philips iE33 system with an L11-3 probe (Philips Tokyo, Japan). IMT was defined as the distance from the leading edge of the first echogenic line to that of the second as previously described.[12] The first line represents the lumen–intima interface, and the second line represents the collagen-containing upper layer of the adventitia. The carotid artery was scanned bilaterally in longitudinal and transverse planes. Maximal IMT (max-IMT) is defined as the highest IMT of several sites in bilateral carotid arteries.[13,14] Mean-IMT was measured at the site of max-IMT and at two other points, 1 cm upstream and 1 cm downstream from this site, and these determinations were averaged.[12,14] All measurements were performed by a

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single expert sonographer who was blinded to the patient's clinical details. The reproducibility of the IMT was evaluated by taking two measurements 2 weeks apart on six volunteers, obtaining an intraclass correlation of 0.98.

Measurement of clinical parameters

Venous blood samples were obtained for chemical analysis after an overnight fast. Plasma omentin levels were determined with omentin enzyme-linked immunosorbent assay kit (Bio Vendor, Candler, NC, USA). Heparin was used for plasma sampling for measurement of omentin. The intra-assay and inter-assay coefficients of variation of this kit were 4.1 and 4.8%, respectively. Adiponectin level was determined with the use of a latex turbidometric immunoassay (Otsuka Pharmaceutical, Tokushima, Japan). Standard assays were used to measure glucose, hemoglobin A1c, insulin, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, creatinine and high-sensitive C-reactive protein levels. Hemoglobin A1c levels were expressed as Japan Diabetes Society values. After an appropriate rest of 10 min, sitting blood pressure was measured. Body mass index was calculated as the ratio of weight to squared height. Estimated glomerular filtration rates (eGFR) were calculated by the Simplified Modification of Diet in Renal Disease equation for Japanese.

Table 1 Clinical parameters and their associations with omentin levels

Parameters	*Subjects (n=100)*	*r*	P-*value*
Age (years)	52.7 ± 8.9	−0.093	0.360
BMI (kg m^{-2})	23.4 ± 2.5	**−0.248**	**0.013**
Waist circumference (cm)	84.9 ± 7.0	**−0.200**	**0.046**
Current smokers (%)	35		0.654
Systolic BP (mm Hg)	115.6 ± 16.2	−0.058	0.566
Diastolic BP (mm Hg)	72.4 ± 11.4	−0.066	0.515
Fasting glucose (mmol l^{-1})	5.60 ± 0.3	**−0.245**	**0.014**
Hemoglobin A1c (%)	5.08 ± 0.39	0.065	0.519
Fasting insulin (mmol l^{-1})	6.15 ± 3.6	0.093	0.355
Total cholesterol (mmol l^{-1})	5.33 ± 0.89	−0.089	0.377
LDL cholesterol (mmol l^{-1})	3.22 ± 0.81	−0.130	0.197
HDL cholesterol (mmol l^{-1})	1.49 ± 0.33	0.059	0.561
Triglyceride (mmol l^{-1})	3.24 ± 1.81	−0.034	0.737
Creatinine (μmol l^{-1})	76.0 ± 12.4	**−0.225**	**0.025**
eGFR (ml min^{-1} per 1.73m^2)	76.2 ± 13.3	**0.254**	**0.011**
Log hsCRP (mg dl^{-1})	2.63 ± 0.47	0.073	0.470
Log adiponectin (μg ml^{-1})	0.69 ± 0.02	0.099	0.329
Max-IMT (mm)	0.74 ± 0.16	**−0.269**	**0.007**
Mean-IMT (mm)	0.66 ± 0.13	**−0.250**	**0.012**
Omentin (ng ml^{-1})	486.1 ± 117.9		

Abbreviations: BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rates; HDL, high-density lipoprotein; hsCRP, high-sensitive C-reactive protein; LDL, low-density lipoprotein; Max-IMT, maximal intima-media thickness.
Data are presented as means ± s.e.
Bold values indicate statistical significance.

Statistical analysis

Results are presented as mean ± s.e. for continuous variables. The correlations of omentin levels with the indicated parameters were investigated by single regression analysis. Single and multiple regression analyses were performed to assess the correlations of the indicated parameters to max-IMT and mean-IMT. A value of $P<0.05$ was considered significant. All analyses were performed using JMP (version 6.03; SAS Institute, Cary, NC, USA).

RESULTS

Clinical characteristics of the study population are shown in Table 1. Mean values of body mass index, blood pressure, hemoglobin A1c, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride and creatinine were within the normal range. Max-IMT and mean-IMT were 0.74 ± 0.16 and 0.66 ± 0.13 mm, respectively. Mean plasma omentin level was 486.1 ± 117.9 ng ml^{-1}. All subjects had no plaque formation in carotid artery. Circulating omentin levels correlated negatively with body mass index, waist circumference, fasting glucose and creatinine, and positively with eGFR (Table 1). Plasma omentin levels inversely correlated with max-IMT and mean-IMT (Table 1 and Figure 1).

Results of regression analysis for max-IMT and mean-IMT are shown in Table 2. Single regression analysis for max-IMT demonstrated that age positively correlated with max-IMT and that eGFR, and omentin levels negatively correlated with max-IMT. Multiple regression analysis with age, smoking status, systolic blood pressure, fasting glucose, total cholesterol, eGFR and omentin revealed that age and omentin levels significantly associated with max-IMT. Single regression analysis for mean-IMT revealed that age and fasting glucose positively correlated with mean-IMT, and that eGFR and omentin levels inversely correlated with mean-IMT. Multiple regression analysis with age, waist circumference, smoking status, fasting glucose, eGFR and omentin revealed that age and omentin levels significantly associated with mean-IMT.

DISCUSSION

This study demonstrated that plasma levels of omentin, together with age, are independently associated with carotid IMT in apparently healthy subjects. Low levels of circulating omentin are shown to associate with type 2 diabetes.[8] It has also been reported that glucose treatment reduces omentin expression and secretion in omental fat tissue explants.[10] Consistent with these observations, our present data

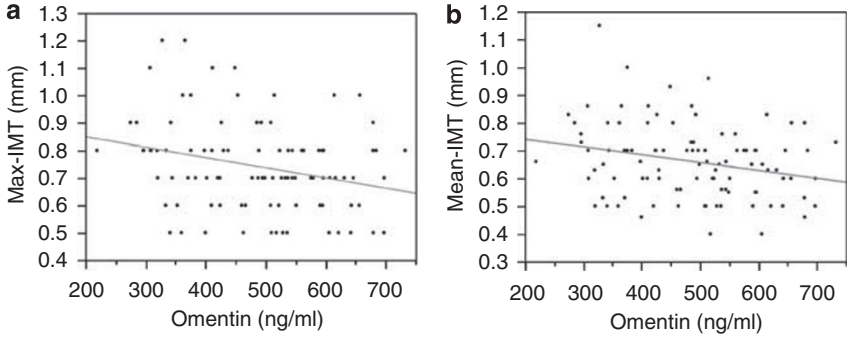

Figure 1 Correlation of plasma omentin levels with maximal intima-media thickness (max-IMT) (**a**) and mean-IMT (**b**). Plasma omentin concentrations were measured by an enzyme-linked immunosorbent assay system. A full color version of this figure is available at the *Hypertension Research* journal online.

Table 2 Correlation with carotid IMT

	Max-IMT				*Mean-IMT*			
	Single		*Multiple*		*Single*		*Multiple*	
	r	P-*value*	F	P-*value*	*r*	P-*value*	F	P-*value*
Age (years)	**0.484**	**<0.001**	**22.20**	**<0.001**	**0.490**	**<0.001**	**21.50**	**<0.001**
BMI ($kg\,m^{-2}$)	0.027	0.787			0.104	0.304		
Waist circumference (cm)	0.094	0.350			0.177	0.078	0.511	0.477
Current Smokers (%)		0.382	1.577	0.213		0.539	1.273	0.262
Systolic BP (mm Hg)	0.168	0.094	0.094	0.760	0.129	0.199		
Diastolic BP (mm Hg)	0.131	0.194			0.120	0.233		
Fasting glucose ($mmol\,l^{-1}$)	0.187	0.063	0.149	0.700	**0.211**	**0.035**	0.190	0.664
Hemoglobin A1c (%)	0.125	0.216			0.067	0.506		
Fasting insulin ($mmol\,l^{-1}$)	−0.074	0.462			−0.015	0.880		
Total cholesterol ($mmol\,l^{-1}$)	0.167	0.097	0.694	0.407	0.119	0.238		
LDL cholesterol ($mmol\,l^{-1}$)	0.153	0.133			0.137	0.174		
HDL cholesterol ($mmol\,l^{-1}$)	−0.042	0.681			−0.066	0.512		
Triglyceride ($mmol\,l^{-1}$)	0.053	0.602			0.056	0.583		
Creatinine ($\mu mol\,l^{-1}$)	0.070	0.487			0.114	0.260		
eGFR ($ml\,min^{-1}$ per $1.73m^2$)	**−0.216**	**0.031**	0.045	0.833	**−0.268**	**0.007**	0.319	0.574
Log hsCRP ($mg\,dl^{-1}$)	−0.106	0.295			−0.005	0.958		
Log adiponectin ($\mu g\,ml^{-1}$)	0.119	0.239			0.026	0.797		
Omentin ($ng\,ml^{-1}$)	**−0.269**	**0.007**	**5.562**	**0.021**	**−0.250**	**0.012**	**4.003**	**0.048**

Abbreviations: BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rates; HDL, high-density lipoprotein; hsCRP, high-sensitive C-reactive protein; LDL, low-density lipoprotein.
Multiple model includes smoking status and all variables at baseline with $P<0.1$ by single analysis.
Bold values indicate statistical significance.

showed that plasma omentin levels negatively associated with glucose levels. Our data also demonstrated that circulating omentin levels correlated inversely with creatinine levels, and positively with eGFR. Because glucose intolerance and renal dysfunction are shown to strongly associate with increased IMT,[15,16] it is reasonable that these disease conditions could affect circulating omentin levels. However, the present study suggests that omentin levels are predictive of carotid IMT, independent of glucose levels and renal function.

A recent report showed that omentin suppresses tumor necrosis factor-stimulated cyclooxygenase-2 expression in cultured endothelial cells through its ability to reduce c-Jun N-terminal kinase activation,[17] suggesting that omentin attenuates vascular inflammation. Omentin is also shown to promote endothelial nitric oxide synthase activation in rat aorta and endothelial cells.[17,18] It is well known that endothelial nitric oxide synthase has a protective role in the control of various vascular diseases including atherosclerosis.[19] These findings suggest that omentin displays anti-atherogenic properties.

It has been suggested that omentin levels positively correlate with endothelium-dependent vasodilation.[9] The current cross-sectional study demonstrated the negative association between plasma levels of omentin and carotid IMT. Accumulating evidence suggests that measurement of endothelial function and carotid IMT can be predictive of cardiovascular and stroke events.[12,20,21] At this time, it remains unclear whether omentin acts as a predictive factor for the vascular outcomes, and this analysis requires future prospective studies.

This study has several limitations. The sample size was relatively small. This study population contained only apparently healthy males. A previous report showed that gender difference is observed in circulating omentin concentration.[7] It has also been shown that carotid IMT is generally greater in men than in women.[14] Although it is well established that plaque thickness is a predictor of cardiovascular risk,[12] no plaque in carotid artery was observed in apparently healthy subjects in this study. Thus, future studies are needed to confirm our results in a larger unselected general population.

In conclusion, the present study indicates that circulating level of omentin is a novel indicator of carotid IMT, and that measurement of omentin levels may be valuable for evaluation of carotid IMT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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