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Omentin-1 and risk of myocardial infarction and stroke: Results from the EPIC-Potsdam cohort study

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ABSTRACT

Background and aims: The recently identified adipokine omentin-1 is inversely associated with body fatness, metabolic syndrome and cardiovascular disease (CVD) in cross-sectional analyses. However, prospective data on the association between plasma omentin-1 levels and future risk of CVD are lacking. The aim of the study was to investigate the relationship between omentin-1 and incident myocardial infarction (MI) and stroke.
Methods: We conducted a case-cohort study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort comprising a subsample of 2084 participants, including 50 CVD cases and 350 external incident CVD cases (mean follow-up of 8.2 ± 1.6 years). Prentice modified Cox regression adjusted for established CVD risk factors was used to estimate associations between omentin-1 and risk of MI and stroke, interactions were tested with cross-product terms.
Results: After multivariable adjustment, omentin-1 was not significantly associated with risk of MI (HR per doubling omentin-1:1.17; 95%-CI:0.79–1.72; $p = 0.43$), but with higher risk of stroke (HR per doubling omentin-1:2.22; 95%-CI:1.52–3.22; $p < 0.0001$). In subgroup analyses, associations between omentin-1 and stroke risk were generally stronger in lower versus higher CVD risk groups. For example, risk of stroke was stronger in participants without metabolic syndrome (HR per doubling omentin-1:2.58; 95%-CI:1.64–4.07; $p < 0.0001$) compared to those with metabolic syndrome (HR per doubling omentin-1:1.21; 95%-CI:0.59–2.50; $p = 0.60$) (p for interaction = 0.05). Similar interactions were observed when participants were classified in low or high risk groups according to waist circumference, triglyceride, hsCRP or adiponectin levels.
Conclusions: Omentin-1 concentrations may be related to increased stroke risk. This association is stronger in metabolically healthy individuals.

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1. Introduction

Omentin-1, also referred to intelectin-1 or intestinal lactoferrin receptor ITLN1, was discovered as novel fat depot-specific secretory protein from a human omental fat cDNA library [1]. This adipokine is mainly expressed in visceral adipose stromal vascular cells, rather than in subcutaneous adipose, but also in lung, heart, placenta, and ovary tissue [1,2]. Omentin occurs in two highly homologous isoforms of omentin-1 and omentin-2, but omentin-1 has been shown to be the major circulating isoform in human plasma [3,4]. Recent studies have observed that omentin-1 is inversely related to body mass index and waist circumference [3]. Positive associations with adiponectin and high density lipoprotein (HDL) cholesterol were also reported [3]. Based on these reported relationships, a possible protective role of omentin-1 in the pathogenesis of atherosclerosis and risk of CVD has been hypothesized.

However, to date, mainly cross-sectional studies investigated the relationship between omentin-1 and cardiovascular endpoints, often performed in patients with preexisting diseases [4,5]. For example Lui et al. found an inverse association between omentin-1 levels and carotid artery intima-media thickness in patients with metabolic syndrome (MetS) [4]. Greulich et al. suggested that decreased omentin-1 levels were associated with cardiovascular dysfunction in patients with type 2 diabetes [5]. Moreover, patients with coronary artery disease (CAD) had lower omentin-1 levels [6–10].

Prospective studies investigating the relationship between omentin-1 and CVD incidence in apparently healthy participants are still missing. Therefore, our study aimed to investigate the longitudinal association between circulating omentin-1 concentrations and risk of myocardial infarction (MI) and stroke in apparently healthy men and women of the EPIC-Potsdam study.

2. Materials and methods

2.1. Study population

The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study consists of 16 644 women and 10 904 men recruited between 1994 and 1998 from the general population in Potsdam and surroundings [11].

About every 2 years, information on incident diseases was collected. To identify potential CVD cases several sources were used: self-report, death certificate or linkage with hospital information system. To increase sensitivity, the questionnaire included additional questions about typical stroke symptoms [12–14]. All identified potential CVD events were ascertained by study physicians, in cooperation with the patients' attending physicians and hospitals, who provided a detailed medical verification of self-reports and death certificates by clinical records according to the World Health Organization Monitoring of Trends and Determinants in Cardiovascular Disease criteria [15]. According to the International Statistical Classification of Diseases, 10th Revision (ICD-10), cases were classified as incident MI (ICD-10 I21), ischemic stroke (IS) (ICD-10 I63.0 to I63.9), hemorrhagic stroke (ICD-10 I60.0 to I61.9) or undetermined stroke (ICD-10 I64.0-I64.9) [16]. All verified incident cases of MI and stroke up to December 2008 were included.

A case-cohort design was applied, using all incident CVD cases and a subcohort of 2500 individuals randomly drawn from all participants of the EPIC-Potsdam study, who provided blood (n = 26 444). This design enables efficient analyses according to time and costs, whereas the results are expected to be generalizable without the need to measure biomarker levels in the entire cohort [17]. After exclusion of individuals with prevalent stroke or MI (n = 85), missing follow-up data (n = 53), inappropriate blood sample (n = 213), missing covariates (n = 107) and unreliable omentin-1 measurements (n = 86), the final study population consisted of a subcohort of 2084 participants, including 50 CVD cases, and 350 verified external incident CVD cases that occurred during 8.2 ± 1.6 years of follow-up (Fig. 1). Participants gave their written informed consent, and study procedures were approved by the Ethics Committee of the Medical Association of the State of Brandenburg.

2.2. Assessment of risk factors and covariates

Lifestyle characteristics, including physical exercise, education and smoking history, were documented at baseline by trained interviewers during a computer-assisted interview [11]. History of hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, self-reports or use of antihypertensive medication. The history of diabetes was evaluated by the study physician using information on self-reported diagnosis, medication records and dieting behavior. Dietary habits including alcohol consumption were assessed by a validated food frequency questionnaire [11].

2.3. Blood collection and laboratory analysis

All markers were measured in citrate plasma. Biomarkers other than omentin-1 were determined at the Department of Internal Medicine, University of Tübingen. Plasma levels of high-density lipoprotein (HDL) cholesterol, total cholesterol, triglycerides, high-sensitivity C-reactive protein (hsCRP) were measured with the automatic ADVIA 1650 analyzer (Siemens Medical Solutions, Erlangen, Germany) in 2007. Adiponectin was measured with an enzyme-linked immunosorbent assay from Linco Research, St Charles, Missouri in 2008.

Plasma omentin-1 concentrations were measured with a sandwich ELISA by Biovendor (Brno, Czech Republic) at the Institute for Clinical Chemistry and Pathobiochemistry, OvGU, Magdeburg, Germany (intra-assay coefficients of variation between 3.2% and

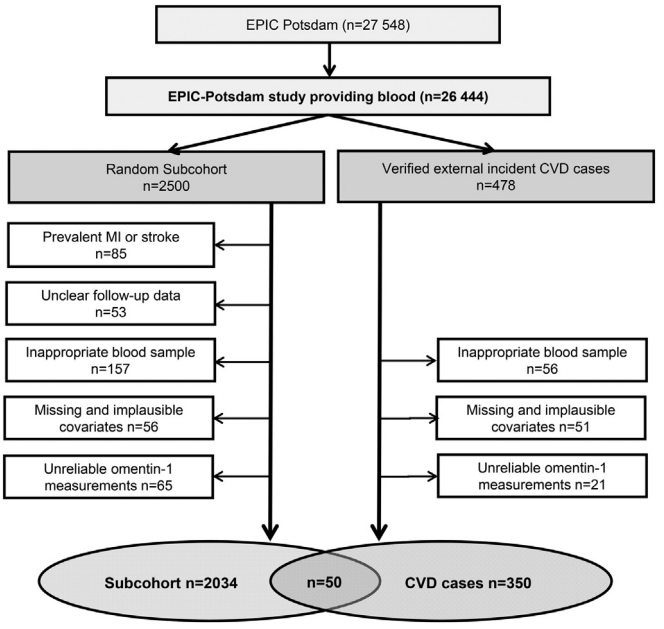

Fig. 1. Flow diagram. Flow diagram for the exclusion criteria indicating the number of subjects excluded and those remaining for the main analysis.

4.1%, inter-assay coefficients of variation between 4.4% and 4.8%) with a limit of detection of 0.5 ng/ml, according to the manufacturer.

2.4. Statistical analysis

A multivariable adjusted analysis of covariance was performed to investigate associations between omentin-1 concentrations and several cardiovascular risk factors across quartiles of omentin-1 concentrations within the subcohort.

Prentice modified Cox proportional-hazards regression was used to estimate the associations between omentin-1 and risk of MI, stroke or its subtypes. Age was used as underlying time axis with "entry time" defined as age at baseline and "exit time" as age at diagnosis of MI or stroke or censoring. Hazard ratios (HR) were adjusted for sex and age (Model 1), waist circumference, smoking, physical activity, education, alcohol consumption, prevalent hypertension, prevalent diabetes, HDL-cholesterol, total cholesterol, triglycerides and hsCRP (Model 2), and adiponectin (Model 3). Alcohol consumption, triglycerides, hsCRP and adiponectin were natural log transformed. For linear relationship, on a continuous scale, omentin-1 was base 2 logarithm transformed, enabling the interpretation of increasing CVD risk per doubling of omentin-1 values. The risk of MI and stroke was furthermore evaluated according to quartiles of omentin-1 using the first quartile as reference.

Competing risk analysis, as described by Lunn and McNeil [18], was applied to test whether the associations of omentin-1 differed between MI and stroke, and additionally between ischemic and hemorrhagic stroke.

Possible nonlinear relationships were examined with restricted cubic splines (RCS) with five knots at the 5th, 35th, 50th (reference), 65th and 95th percentile of omentin-1 in the fully adjusted model. The Wald chi-square test was used to select the best-fit model.

Effect modifications between selected CVD risk factors (dichotomous) and omentin-1 (continuous) in relation to MI and stroke risks were tested with cross-product terms in model 3. Stratified analyses were performed according to established cutoff points: waist circumference (women $</\geq$ 88 cm; men $</\geq$ 102 cm) [19], HDL-cholesterol (women $</\geq$ 1.29 mmol/l; men $</\geq$ 1.04 mmol/l) [19], triglycerides ($</\geq$ 1.69 mmol/l) [19], hsCRP ($</\geq$ 1.0 mg/l) [20]. For adiponectin the sex-specific median from the subcohort was used (women $</\geq$ 9.14 μg/ml; men $</\geq$ 5.98 μg/ml). Metabolic syndrome was defined using the American Heart Association/National Heart, Lung and Blood Institute criteria [19].

The proportional hazard assumption was tested by a Kolmogorov-type supremum test. No violation was found. A p-value <0.05 was considered to be statistically significant, for interactions p-value was increased to 0.10. Sensitivity analyses were performed after exclusion of first 2 years of follow-up, to account for the latency period between pathology and clinical diagnosis. Additionally Cox proportional-hazards regression analyses were applied to estimate the associations between omentin-1 and risk of MI and stroke with further adjustment of antidiabetic medication, antihypertensive medication and lipid-lowering medication in model 2 and model 3. All statistical analyses were performed using SAS software, version 9.4 (SAS institute, Cary, N.C., USA).

3. Results

Participants with incident MI or stroke were older and more likely to be male compared to persons without CVD events (Supplemental Table 1). After adjustment for age and sex, persons with CVD events at baseline were also more likely to be smokers and to have a history of diabetes and hypertension compared to those without CVD events (Supplemental Table 1).

Within the subcohort, a cross-sectional multivariable adjusted analysis of covariance across quartiles of omentin-1 showed significant positive associations with age, physical activity, and history of diabetes, HDL-cholesterol, adiponectin, and alcohol consumption, significant inverse associations were observed with waist circumference (Table 1).

Participants in the highest quartile of omentin-1 levels had a significantly increased risk of stroke (Model 3, HR: 2.29; 95%-confidence interval (CI) 1.38–3.79; p linear trend = 0.0003) compared to participants in the lowest quartile (Table 2). Positive associations were also observed when omentin-1 was modelled on a continuous scale (Model 3 per doubling of omentin-1, HR: 2.22; 95%-CI: 1.52–3.22; $p < 0.0001$). Separated analyses showed increased risk of both ischemic and hemorrhagic stroke in the highest versus the lowest quartile of omentin-1 (Table 2). However, no relationship was observed between omentin-1 and risk of MI (Table 2 and Fig. 2).

In a competing risk analysis, a significant difference in the association between omentin-1 and MI versus omentin-1 and stroke was detected ($p = 0.02$). However, no difference was observed in the associations between omentin-1 and ischemic stroke versus omentin-1 and hemorrhagic stroke ($p = 0.43$).

There was no evidence of departure from linearity for the relation between omentin-1 and stroke risk (p for non-linearity = 0.82). We found no significant non-linear associations between omentin-1 and risk of MI (Fig. 2).

Stratified analyses according to CVD risk subgroups showed an association between omentin-1 and increased stroke risk in particular in metabolically healthy individuals. As presented in Table 3, in participants with high adiponectin levels higher omentin-1 levels were related to a higher risk of stroke (HR per doubling omentin-1: 3.52; 95%-CI: 2.08–5.94; $p < 0.0001$), and omentin-1 was not associated with stroke risk in participants with low adiponectin levels (HR per doubling omentin-1: 1.14, 95%-CI: 0.65–2.01, $p = 0.65$) (p for interaction = 0.02). Furthermore, risk of stroke was stronger in participants without MetS (HR per doubling omentin-1: 2.58; 95%-CI: 1.64–4.07; $p < 0.0001$) compared to those with MetS (HR per doubling omentin-1: 1.21; 95%-CI: 0.59–2.50; $p = 0.60$) (p for interaction = 0.05). Similar interactions were observed when participants were classified in low or high risk groups according to waist circumference (p for interaction = 0.03), triglyceride (p for interaction = 0.09) or hsCRP (p for interaction = 0.08). According to MI risk, stratified analyses showed a decreased MI risk in participants with MetS and a stronger MI risk in individuals without MetS (p for interaction = 0.02), though both associations were not statistically significant (Supplemental Table 2).

After exclusion of cases that occurred during the first 2 years of follow-up, the hazard ratios of stroke and MI according to omentin-1 concentrations remained unaltered (data not shown). Further, we observed no changes in results with additionally adjustment of antidiabetic medication, antihypertensive medication and lipid-lowering medication in the main multivariable analysis (Model 2 and Model 3, data not shown).

4. Discussion

In this prospective analysis, we observed that higher levels of

Table 1
Characteristics of the subcohort according to omentin-1 quartiles.

Characteristics	Quartiles of omentin-1 in the subcohort[a]				*p* linear trend[c]	*p* linear trend[d]
	Q1	Q2	Q3	Q4		
n	519	525	519	521		
Omentin-1 [ng/ml][b]	286.5 (250.6–308.6)	363.1 (343.4–380.3)	439.6 (420.0–462.8)	569.6 (517.4–642.6)		
Men [%][c]	41.2	36.6	37.7	33.5	0.02	0.1
Age [years][c]	47.4 (46.6–48.1)	48.8 (48.1–49.5)	51.9 (51.1–52.6)	53.9 (53.2–54.6)	<0.0001	<0.0001
Waist circumference [cm]	89.8 (88.8–90.7)	87.4 (86.5–88.3)	86.6 (85.7–87.5)	85.1 (84.2–86.0)	<0.0001	<0.0001
Physical activity, [h/week]	0.88 (0.73–1.03)	0.91 (0.76–1.05)	0.98 (0.83–1.13)	1.26 (1.11–1.41)	0.0005	0.01
Smoking [%]					0.3	0.5
Non-smoker	43.1	43.3	40.7	46.9		
Ex-smoker < 5 years	26.1	27.3	28.4	24.8		
Ex-smoker ≥ 5 years	7.4	6.4	8.7	8.9		
Smoker < 20 cigarettes/day	16.0	14.9	17.2	12.6		
Smoker ≥ 20 cigarettes/day	7.3	8.1	4.9	6.8		
Education [%]					0.7	0.4
Unskilled or skilled	35.4	35.0	35.2	35.4		
Technical College	22.4	23.5	23.5	21.3		
University degree	42.2	41.5	41.2	43.3		
Prevalent diabetes [%]	3.7	3.8	4.6	5.9	0.07	0.0004
Antidiabetic medication [%]	1.6	1.6	3.1	3.2	0.03	0.0004
Prevalent hypertension [%]	50.7	48.7	47.4	49.6	0.6	0.06
Antihypertensive medication [%]	19.4	16.0	16.6	19.0	0.9	0.07
Lipid-lowering medication [%]	4.7	4.4	3.6	4.0	0.5	0.5
Total cholesterol [mmol/l]	5.24 (5.15–5.33)	5.26 (5.17–5.36)	5.26 (5.17–5.35)	5.37 (5.27–5.46)	0.08	0.4
HDL-cholesterol [mmol/l]	1.36 (1.32–1.39)	1.39 (1.36–1.42)	1.41 (1.38–1.44)	1.53 (1.50–1.56)	<0.0001	0.03
Triglyceride [mmol/l]	1.64 (1.55–1.73)	1.53 (1.44–1.62)	1.66 (1.57–1.75)	1.47 (1.37–1.56)	0.0003	0.7
hsCRP [mg/l]	2.57 (2.26–2.89)	1.67 (1.37–1.97)	1.89 (1.59–2.20)	1.64 (1.33–1.95)	<0.0001	0.06
Adiponectin [μg/ml]	6.99 (6.67–7.32)	7.78 (7.46–8.10)	8.23 (7.90–8.55)	9.30 (8.97–9.63)	<0.0001	<0.0001
Alcohol [g/d]	14.6 (12.9–16.3)	16.5 (14.8–18.2)	16.6 (14.9–18.2)	19.4 (17.7–21.1)	<0.0001	0.0004

[a] All variables were adjusted for sex and age, expressed as adjusted percentage or mean and 95%-CI.
[b] Unadjusted variable, expressed as median and interquartile range.
[c] Adjusted for sex, age; according to examined variable.
[d] Mutually adjusted for sex, age, waist circumference, physical activity, education, smoking, alcohol consumption, prevalent hypertension and diabetes, HDL-cholesterol, total cholesterol, triglycerides, hsCRP and adiponectin according to examined variable.

Table 2
Hazard ratios of MI and stroke according to quartiles and per doubling of omentin-1 levels.

	Quartiles of omentin-1 levels				*p* for trend	Per doubling of omentin-1	
	Q1	Q2	Q3	Q4			*p*-value
Omentin-1 [ng/ml][a]	286.5 (250.6–307.4)	364.2 (344.0–380.5)	439.6 (420.0–462.2)	571.9 (519.5–642.6)			
Subcohort participants (n)	519	525	519	521			
Follow-up time [years]	4245.8	4288.9	4304.1	4234.2			
MI (n = 2267)							
Cases (n)	43	45	54	60			
Sex and age adjusted	Reference	0.99 (0.63–1.54)	0.91 (0.60–1.39)	0.95 (0.62–1.45)	0.80	0.96 (0.68–1.35)	0.79
Model 2[b]	Reference	0.90 (0.56–1.44)	0.97 (0.62–1.51)	1.16 (0.73–1.83)	0.42	1.19 (0.81–1.75)	0.37
Model 3[c]	Reference	0.89 (0.56–1.42)	0.96 (0.61–1.50)	1.13 (0.71–1.79)	0.48	1.17 (0.79–1.72)	0.43
Stroke (n = 2251)							
Cases (n)	24	34	55	85			
Sex and age adjusted	Reference	1.29 (0.75–2.23)	1.73 (1.05–2.84)	2.39 (1.50–3.82)	<0.0001	2.12 (1.54–2.92)	<0.0001
Model 2[b]	Reference	1.26 (0.72–2.20)	1.63 (0.97–2.73)	2.42 (1.47–3.98)	0.0001	2.31 (1.59–3.35)	<0.0001
Model 3[c]	Reference	1.24 (0.71–2.16)	1.58 (0.94–2.66)	2.29 (1.38–3.79)	0.0003	2.22 (1.52–3.22)	<0.0001
Ischemic stroke (n = 2218)							
Cases (n)	19	29	45	68			
Sex and age adjusted	Reference	1.37 (0.75–2.51)	1.73 (1.00–3.02)	2.31 (1.37–3.90)	0.0006	1.96 (1.38–2.79)	0.0002
Model 2[b]	Reference	1.34 (0.72–2.47)	1.59 (0.89–2.85)	2.34 (1.34–4.10)	0.001	2.13 (1.41–3.20)	0.0003
Model 3[c]	Reference	1.32 (0.71–2.43)	1.54 (0.86–2.76)	2.20 (1.25–3.86)	0.003	2.02 (1.34–3.05)	0.0008
Hemorrhagic stroke (n = 2114)							
Cases (n)	4	5	10	15			
Sex and age adjusted	Reference	1.23 (0.33–4.63)	2.19 (0.70–6.90)	3.18 (1.06–9.55)	0.009	2.62 (1.43–4.79)	0.002
Model 2[b]	Reference	1.32 (0.35–4.97)	2.28 (0.74–7.05)	3.46 (1.11–10.8)	0.01	2.85 (1.43–5.68)	0.003
Model 3[c]	Reference	1.30 (0.35–4.91)	2.28 (0.74–7.04)	3.37 (1.08–10.6)	0.01	2.80 (1.39–5.62)	0.004

Hazard ratios and 95%-CI were derived from Cox proportional hazard regression.
[a] Quartiles are based on the distribution of omentin-1 within the subcohort expressed as median and interquartile range.
[b] Additionally adjusted for waist circumference, physical activity, education, smoking, alcohol consumption, prevalent hypertension and diabetes, HDL-cholesterol, total cholesterol, triglycerides and hsCRP.
[c] Additionally adjusted for adiponectin levels.

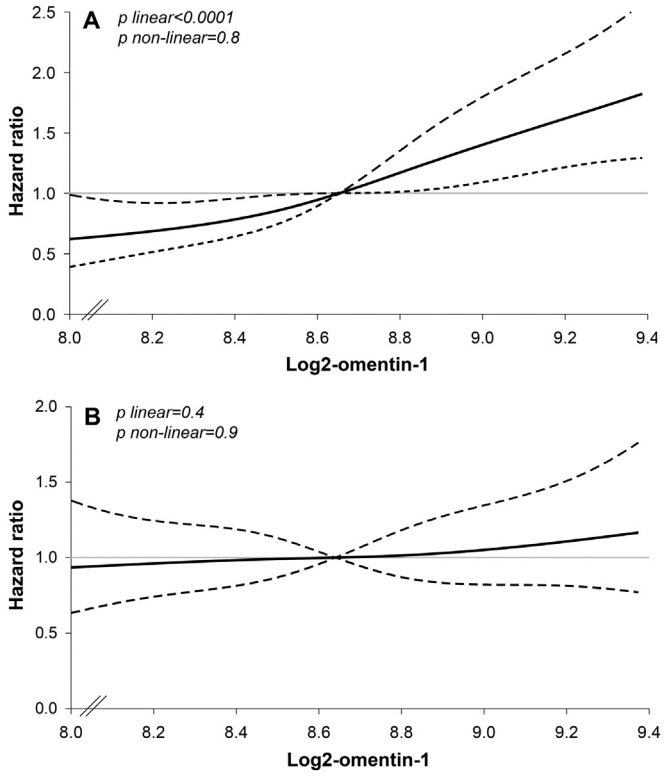

Fig. 2. Hazard ratio curves. (A) Hazard ratio curves for the association between omentin-1 levels and the risk of stroke. (B) Hazard ratio curves for the association between omentin-1 levels and the risk of MI. The solid lines indicate hazard ratios of stroke or MI as obtained by restricted cubic spline Cox regression with knots placed at fixed values (5th, 35th, 65th and 95th percentile of the distribution of omentin-1 in the entire case-cohort). The reference was set at 50th percentile. Dashed lines indicate 95%-CI. Hazard ratios and 95%-CI bands are adjusted for age, sex, waist circumference, physical activity, education, smoking, alcohol consumption, prevalent hypertension and diabetes, HDL-cholesterol, total cholesterol, triglycerides, hsCRP, adiponectin. P for nonlinearity was computed by Wald chi-square test.

omentin-1 were significantly associated with a higher risk of stroke in metabolically healthy participants with normal waist circumference, low levels of triglyceride and hsCRP, high adiponectin levels and no MetS. On the contrary, omentin-1 was not significantly related to risk of MI.

To our knowledge, this is the first prospective study investigating potential associations between omentin-1 and risk of stroke and MI in apparently healthy middle aged men and women. So far, only two small prospective studies investigated the association between omentin-1 and cardiovascular endpoints in patients with heart disease, providing conflicting findings [21,22]. In line with our study, Saely et al. reported an association between higher plasma omentin-1 levels and higher risk of cardiovascular events in 295 CAD patients [22,23]. In contrast, in a study comprising 136 patients with heart failure, Narumi and colleagues observed an association between lower omentin-1 levels and higher risk of cardiac death or re-hospitalization [21].

Results from cross-sectional studies suggested omentin-1 as a potential cardio-protective factor, inversely related to cardiovascular risk factors and intermediates. Interestingly, cross-sectional analyses from our study are in line with previous studies providing additional evidence regarding cross-sectional associations between omentin-1 and lifestyle factors, blood lipids and adiponectin. Our study supports recently reported inverse association between omentin-1 and waist circumference [2,3,5]. Moreover, we observed a positive association between omentin-1 and HDL-cholesterol [3,5,10,24,25] as well as a positive association between omentin-1 and adiponectin consistent with previous studies [3,24,26]. In line, also relations between omentin-1 and clinical intermediate cardiovascular phenotypes were observed. Cross-sectional studies including patients with type 2 diabetes or MetS, observed inverse associations between omentin-1 and arterial stiffness/carotid plaque as well as left ventricular diastolic function [5,27]. Moreover, Lui et al. reported an inverse relation between omentin-1 levels and carotid artery intima-media thickness in MetS patients [4]. Nevertheless, these findings were observed in studies comprising low number of participants with existing diseases. Interestingly, restricting our results to a subgroup of

Table 3
Multivariable adjusted hazard ratios for stroke per doubling of omentin-1 for subgroups.

Group	Cases	Subcohort[a]	*p* for interaction[b]	Hazard ratio (95%-CI)[c]	*p*-value
Sex			0.68		
Men	114	754		2.65 (1.55–4.52)	0.0004
Women	84	1299		1.90 (1.06–3.40)	0.03
Waist circumference [cm]			0.03		
< 102 men, < 88 women	134	1569		2.59 (1.63–4.11)	<0.0001
≥ 102 men, ≥ 88 women	64	484		1.76 (0.82–3.77)	0.15
Metabolic syndrome[d]			0.05		
No	128	1649		2.58 (1.64–4.07)	<0.0001
Yes	70	402		1.21 (0.59–2.50)	0.60
HDL-cholesterol [mmol/l]			0.49		
< 1.04 men, < 1.29 women	55	490		2.76 (1.23–6.17)	0.01
≥ 1.04 men, ≥ 1.29 women	143	1563		2.16 (1.38–3.38)	0.0007
Triglycerides [mmol/l]			0.09		
< 1.69	123	1480		3.16 (1.90–5.25)	<0.0001
≥ 1.69	75	573		1.28 (0.73–2.26)	0.39
Adiponectin [μg/ml]			0.02		
< 5.98 men, < 9.14 women	91	1031		1.14 (0.65–2.01)	0.65
≥ 5.98 men, ≥ 9.14 women	107	1022		3.52 (2.08–5.94)	<0.0001
hsCRP [mg/l]			0.08		
< 1.0	90	1165		2.98 (1.70–5.21)	0.0001
≥ 1.0	108	888		1.49 (0.83–2.66)	0.18

[a] Subcohort without internal cases.
[b] *p*-values for interaction were calculated using dichotomous variables and log-transformed omentin-1 levels.
[c] Adjusted for age, sex, waist circumference, physical activity, education, smoking, alcohol consumption, prevalent hypertension and diabetes, HDL-cholesterol, total cholesterol, triglycerides, hsCRP, adiponectin.
[d] n = 2249 due to MetS missing values.

participants with MetS we also observed an inverse, though non-significant, association between omentin-1 and risk of MI. Therefore we may hypothesize that the role of omentin-1 in metabolic regulation probably systematically differs in participants with pre-existing metabolic disease as compared to those apparently healthy. However, the suggested protective effect was not observed in stratified analysis regarding risk of stroke, although a lower risk in participants with MetS was observed, compared to participants without MetS.

With regard to stroke risk, we observed a significant positive interaction between omentin-1 and waist circumference as well as between omentin-1 and adiponectin. Interestingly, adiponectin and omentin-1 share the same pattern of physiological effects i.e. both are downregulated in obesity [3,28], both have been proposed to have a beneficial effect on glucose metabolism and insulin sensitivity [1,29], and both were discussed as cardio-protective adipokines [28,30]. However, the association between omentin-1 and stroke risk was particularly strong among individuals with high adiponectin levels compared to those with low levels. Therefore, a complex molecular interplay between omentin-1 and adiponectin in relation to risk of stroke might be hypothesized. Yet, the available evidence is insufficient to infer a possible mechanism explaining the observed interaction between omentin-1 and adiponectin. To date, it is uncertain whether the regulation of omentin-1 may be dependent on adiponectin or *vice versa* [3]. So far, only Herder et al. have hypothesized that circulating omentin-1 levels regulate adiponectin levels [24].

Up to date, experimental studies have suggested omentin-1 as a potential cardio-protective factor [31–37]. Yamawaki et al. have shown that omentin-1 may enhance vasodilation in vascular endothelial cells vessels through endothelium-derived nitric oxide [31]. In addition, omentin-1 has been suggested to increase AMP-activated protein kinase (AMPK) phosphorylation, and to promote endothelial cell function e.g. enhancement of endothelial cell differentiation [32], protection of cardiac myocytes from apoptosis during ischemia [33] and attenuate neointimal formation after arterial injury in vivo [34]. Furthermore, omentin-1 may exert anti-inflammatory functions via AMPK signaling pathway [35–37].

However, as already mentioned in line with our study results, recently another prospective study by Sealy et al. refuted the suggested cardio-protective associations and in addition demonstrates that omentin-1 strongly predicts cardiovascular events [22,23]. Yet, the reasons for such discrepant findings are unclear, demonstrating that the biological action of omentin-1 in the cardiovascular biology is hardly understood. So far, we might speculate, somehow, an omentin-1-related stroke risk depended from different metabolic conditions e.g. high or low level of adiponectin, triglycerides or hsCRP. Competition for possible shared signal pathways with different signaling efficiencies would in principle agree with our observations. We suspect a molecular interplay between omentin-1 and other CVD risk factors to be of complex nature and to be probably altered in the pathophysiological context.

However, given the strong associations between higher omentin-1 levels and stroke risk on the one hand and the non-significant relation with MI on the other hand, intense research and well-designed experiments are needed, addressing the biological processes of omentin-1 with the risk of different major cardiovascular endpoints under different metabolic conditions.

Strengths of the current study include the prospective study design, the availability of high quality data as a result of the standardized procedures enabling us to adjust for a large variety of potential confounders, the high follow-up response rate, and the rigorous case validation. Nevertheless, the present results are limited to middle aged Caucasian participants and might be not generalized to other ethnic groups. The competing risk analyses revealed no significant differences in the association omentin-1 and ischemic stroke compared to hemorrhagic stroke, but based on the limited number of cases, in particular for hemorrhagic stroke, a chance finding cannot be completely ruled out. Furthermore, the present findings are based on one single baseline measurement of omentin-1 levels with long-term sample storage. Nevertheless, EPIC-Potsdam provides high quality blood samples, because of the highly standardized processes for sample collection and storing. Plasma samples used for omentin-1 assessment were stored at −80 °C in freezers, a temperature that guarantees the stability of samples for long periods of time for a wide range of analytes, including highly labile molecules such as cytokines [38,39]. Moreover, we observed excellent reliability of omentin-1 over time indicated by intraclass correlation coefficient (ICC) of 0.83 (95%-CI 0.78–0.87), which was very similar to the ICC reported from a previous study [40], suggesting that a single measurement may provide reliable risk estimates [41].

In conclusion, the current study observed that high plasma omentin-1 concentrations were associated with a higher risk of stroke in particular in participants with normal waist circumference, low levels of triglyceride and hsCRP, high adiponectin levels or no metabolic syndrome. Further studies are needed to explain the different role of omentin-1 under different metabolic conditions.

Conflict of interest

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2016.06.003.

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