Association of Circulating Chemerin With Subclinical Parameters of Atherosclerosis
Results of a Population-Based Study

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Objective—Chemerin has been shown to be associated with inflammation and metabolic syndrome, which are in turn leading risk factors for atherosclerosis. A few clinical studies have concentrated on the role of chemerin in atherosclerosis but revealed divergent findings. Therefore, we aimed to investigate the association of plasma chemerin levels with different subclinical measurements of atherosclerosis in a population-based sample.

Approach and Results—Linear and logistic regression models with different atherosclerotic parameters as subclinical outcomes were applied to analyze data from 4003 subjects of the SHIP (Study of Health in Pomerania). After adjustment for metabolic and inflammatory parameters, these models revealed no association of chemerin with carotid intima-media thickness, carotid plaque, or carotid stenosis but a significant inverse association between chemerin and ankle-brachial index. In detail, logistic regression analysis showed that a 25-ng/mL increase in chemerin was associated with a 30% higher odd (95% confidence interval, 1.20–1.41) of having an ankle-brachial index value below the 25th age- and sex-specific quartile.

Conclusions—Our analyses revealed a modest inverse association between chemerin and ankle-brachial index that remained consistent after adjustment for metabolic and inflammatory parameters. The association of chemerin with carotid intima-media thickness, carotid plaque, or carotid stenosis was not significant after adjustment for the same confounder set. The investigated subclinical atherosclerotic parameters are representative for the atherosclerotic burden of different arterial regions and different disease stages. Thus, our results might suggest that the value of chemerin as a marker of higher atherosclerotic risk differs depending on the affected arterial region and disease stage.

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Key Words: adipokines ■ ankle-brachial index ■ atherosclerosis ■ carotid intima-media thickness ■ chemerin ■ inflammation ■ risk factors

Obesity is an important risk factor for atherosclerosis, which causes vascular complications by aggravating blood pressure, insulin resistance, lipid metabolism, and systemic inflammation.[1] Furthermore, obesity also plays a direct role in atherosclerotic processes because adipokines—bioactive molecules that are secreted by adipose tissue—influence the function of endothelial cells, vascular smooth muscle cells, and macrophages in the vessel wall.[1,2] It is likely that an adipokine that influences or just documents the atherosclerotic process might serve as a useful therapeutic agent or biomarker and, therefore, might contribute to the prediction or prevention of serious cardiovascular morbidities in later life.

The adipokine chemerin, that is encoded by *RARRES2* (retinoic acid receptor responder 2), exerts its biologically effects mainly via the CMKLR1 (chemokine-like receptor 1).[3] In humans, chemerin and CMKLR1 mRNA are abundantly expressed in white adipose tissue.[4] Furthermore, CMKLR1 is highly expressed by several immune cells, including dendritic cells, monocytes, macrophages, and natural killer cells.[3] Experimental research has demonstrated a role of chemerin in inflammation by highlighting its function in the recruitment of these immune cells to lymphoid organs and sites of injury.[5] Furthermore, circulating chemerin levels have been associated with parameters of inflammation, obesity, and metabolic syndrome.[6] In light of these findings, previous studies increasingly focused on the potential role of chemerin in the development of atherosclerosis. Several experimental examinations have suggested inflammatory functions of chemerin during early stages of the atherosclerotic process.[7–11] But it is currently still not clear whether an association between human circulating chemerin and atherosclerosis exists. Some case-control studies have shown that higher circulating chemerin levels are associated

Nonstandard Abbreviations and Acronyms	
ABI	ankle-brachial index
BMI	body mass index
cIMT	carotid intima-media thickness
CMKLR1	chemokine-like receptor 1
HbA1c	glycohemoglobin
HDL	high-density lipoprotein
hsCRP	high-sensitivity C-reactive protein
LDL	low-density lipoprotein
MMP	matrix metalloproteinase
NASCET	North American Symptomatic Endarterectomy Trial
RARRES2	retinoic acid receptor responder 2
SHIP	Study of Health in Pomerania

with prevalent atherosclerosis (ie, carotid plaque,[12] carotid stenosis,[13–16] and acute coronary syndrome[17]) and with subclinical markers of atherosclerosis like carotid intima-media thickness (cIMT), brachial pulse wave velocity, or ankle-brachial index (ABI),[18–20] whereas others did not confirm these observations.[21–26]

Here, we provide the first analyses of a well-characterized, large population-based sample to investigate the association of plasma chemerin levels with a set of subclinical measurements of atherosclerosis, including cIMT and ABI, as well as the presence of carotid plaque and carotid stenosis. We hypothesize that high plasma chemerin levels are associated with subclinical atherosclerotic lesions.

Materials and Methods

All data and supporting materials have been provided with the published article.

Study Population

The SHIP (Study of Health in Pomerania) is a population-based project in West Pomerania—a rural, sparsely populated region in the northeast of Germany.[27] To date, the overall research project consists of 2 separate cohorts (SHIP and SHIP-TREND). The present study is based on data from the SHIP-TREND cohort. In SHIP-TREND, a stratified random sample of 8826 people, aged 20 to 79 years, was drawn from local population registries. The baseline examinations were conducted between 2008 and 2012. Four thousand four hundred twenty of the invited adults chose to participate (50.1% response). The study followed the principles of the Declaration of Helsinki[28] and was approved by the ethics committee of the University of Greifswald. SHIP data are publically available for scientific and quality control purposes, and data usage can be applied for via www.community-medicine.de.

Of the 4420 subjects available for analyses, pregnant women (n=10) and subjects with severely restricted renal function, defined by an estimated glomerular filtration rate <30 mL/min per 1.73 m^2 (n=18), were excluded from the study population. Furthermore, we excluded subjects with missing data in chemerin levels (n=101) or in any of the confounding or outcome variables (n=242). Observations with plasma chemerin levels that were >3 SDs above the corresponding sex-specific population average were considered as outliers and, therefore, excluded from the data set (n=46). The final study population consisted of 4003 subjects (48.7% women) aged 20 to 84 years. Among these subjects, 62.15% participated in an external ABI examination, resulting in a subsample that includes 2488 subjects.

Measurements

Data on age, sex, sociodemographic characteristics, and medical histories were obtained by standardized computer-assisted personal interviews. Smoking status was categorized into current, former, or never smoker. Anthropometric measurements were taken during the physical examination according to the World Health Organization standards. The weight and height of each subject was used to calculate its body mass index (BMI, kg/m^2). Waist circumference was measured to the nearest 0.1 cm using an inelastic tape midway between the lower rib margin and the iliac crest in the horizontal plane with the subject standing comfortably with weight distributed evenly on both feet. The measurement was taken at the level of the narrowest part of the waist. After a 5-minute resting period, blood pressure was measured 3× on seating subjects using a digital blood pressure monitor (HEM-705CP; Omron, Tokyo, Japan), with each reading being followed by a further resting period of 3 minutes. The mean of the second and third measurements was taken for these analyses. The use of antihypertensive medication was classified according to self-reported data from the questionnaire.

Blood samples were collected between 7 AM and 1 PM from the cubital vein of mostly fasting subjects in the supine position. Aliquots were prepared for immediate analysis and for storage at −80°C. Total triglycerides, total cholesterol, HDL (high-density lipoprotein) cholesterol, LDL (low-density lipoprotein) cholesterol , and hsCRP (high-sensitivity C-reactive protein) were measured using the Dimension Vista analytic system (Siemens AG, Eschborn, Germany). Lipid-lowering medication was classified according to anatomic, therapeutic, and chemical code (C10). HbA1c (glycohemoglobin) was determined by high-performance liquid chromatography with spectrophotometric detection (Diamat Analyzer; Bio-Rad, Munich, Germany). Serum creatinine concentrations were determined with a modified kinetic Jaffé method (Dade Behring, Inc, Newark). The modification of diet in renal disease formula[29] was applied to calculate the creatinine-based estimated glomerular filtration rate. Circulating chemerin levels were determined in EDTA plasma using a commercially available ELISA technique (Mediagnost Chemerin ELISA E102; Reutlingen, Germany). The interassay coefficients of variation for HbA1c, hsCRP, total triglycerides, creatinine, and chemerin were 3.2%, 3.8%, 4%, 7.4%, 5.8% and 2.4%, 3.6%, 3.4%, 4.8%, 5.5% for low and high concentrations, respectively.

Trained and certified medical assistants examined the extracranial carotid arteries bilaterally with B-mode ultrasonography using a 13-MHz broad-bandwidth linear array transducer (Vivid TMi; GE Medical Systems, Waukesha, WI) following guidelines of detailed standard operating procedures. For the measurement of the cIMT, longitudinal scans from the distal straight portion of both common carotid arteries were recorded. cIMT was assessed on-screen using a semiautomated edge tracking software, which measures the distance between the lumen-intima and media-adventitia interfaces at an arterial segment of 1 cm length located directly proximal to the widening of the artery at the bifurcation. The cIMT was calculated as the average of the mean values of 250 measurement points of each side: (right mean cIMT+left mean cIMT)/2. If only information from 1 side was available, this value was taken as mean value for cIMT. A high cIMT value was defined as a cIMT above the sex- and age-related 75th quartile (n=997). Atherosclerotic plaques were evaluated in cross-sectional and longitudinal scans of the common carotid artery, the carotid bifurcation, the internal carotid artery, and the external carotid artery. Plaques were defined as any focal thickening of the intima-media complex protruding into the vessel lumen or as a focal increase of echogenicity with a homogenously hyperechoic echo texture within an otherwise hypoechoic intima-media complex. Carotid plaque was categorized as being present if at least 1 arterial segment was classified as being affected by plaque. Furthermore, Doppler spectra of the proximal internal carotid artery were recorded, and according to the NASCET (North American Symptomatic Endarterectomy Trial) criteria,[30] a stenosis >20% was defined as a plaque that causes a focal increase of systolic flow velocities.

For ABI measurements, systolic blood pressure was measured with a Doppler ultrasound probe (Dopplex D900; Huntleigh Healthcare, Ltd, Cardiff, United Kingdom) and a blood pressure cuff (Welch Allyn, Inc, Skaneateles Falls, NY) in both arms (brachial artery) and ankles (anterior and posterior tibial artery). Measurements were performed after a rest period of at least 10

minutes and followed detailed standard operating procedures. ABI calculations followed the guidelines of the American Heart Association.[31] The higher value of the anterior and posterior tibial artery for each leg was chosen and divided by the higher value of the right or left brachial artery pressures. Finally, the lower value of the right or left leg ABI was used for statistical analyses. A low ABI was defined as an ABI value smaller than the 25th sex- and age-specific quartile (n=579).

To quantify measurement variability of the presented subclinical parameters of atherosclerosis, we calculated the proportion of variance that is attributable to the medical assistants. As our results showed that for each parameter <2% of the total variance in measurements can be explained by differences because of the medical assistants, we conclude that the influence of the medical assistant on the presented measurements is negligible.

Statistical Analyses

Continuous data are expressed as median (25th–75th quartile), and nominal data are given as percentage. An ANOVA was performed to calculate adjusted means for cIMT and ABI across sex-specific plasma chemerin quartile groups. Further, the influence of plasma chemerin on cIMT and ABI values was assessed by multivariable linear regression models. Sex-specific analyses were not necessary because including interaction terms of chemerin with sex in the regression models revealed no significant effect modifications. Possible nonlinear associations were tested by including restricted cubic splines with 3 knots at the 5th, 50th, and 95th percentile.[32] In none of the models, the likelihood ratio test indicated a significant increase in model fitness if the spline term for chemerin was included. Therefore, we assumed linear associations of chemerin with cIMT and ABI. Regression models were adjusted for age and sex in a first step. To identify possible changes with obesity, other metabolic and inflammatory risk factors, as well as systolic blood pressure and renal function, all models were additionally adjusted for waist circumference, HbA1c, LDL cholesterol, hsCRP, systolic blood pressure, and estimated glomerular filtration rate in a second step. Similarly, logistic regression models were applied to analyze the association of plasma chemerin with the odds of carotid plaque and carotid stenosis, as well as high cIMT and low ABI. Because lipid-lowering and antihypertensive medication are known to influence atherosclerotic disease progression and might also have an influence on circulating chemerin levels, we additionally adjusted our analyses for the use of these medications in a third model that is presented in the online-only Data Supplement.

Because only a subset of our study population participated in ABI measurement, selection bias introduced by missing data is possible. Therefore, we corrected all analyses of ABI measurements by weighting the available values by the inverse probability of being a complete case.[33] The inverse probability weights were derived from a logistic regression model that used participation at the ABI examination as the outcome variable and age, sex, per capita income, highest school degree, hypertension, diabetes mellitus type 2, BMI, total cholesterol, and smoking status as explanatory variables.

In old subjects or those with long-lasting diabetes mellitus, calcified vessels are possible (Monckeberg arteriosclerosis), which can lead to distorted ABI measurements of >1.3.[34] To avoid a potential bias because of high ABI values, we repeated all multivariable analyses after exclusion of individuals with ABI values >1.3 (n=114). In a further sensitivity analysis, we examined the consistency of our results by repeating all analyses after excluding subjects with a known clinical history of cardiovascular diseases (self-reported angina pectoris, myocardial infarction, atrial fibrillation, stroke, or heart failure; n=589). Moreover, we repeated all analyses in subsamples of normal-weight (18.5≤BMI<25; n=1127), overweight (25≤BMI<30; n=1611), and obese (BMI≥30; n=1252) subjects to fully account for the level of adiposity when examining the association of chemerin with subclinical atherosclerotic parameters. The group of underweight subjects (BMI, <18.5) was not taken into account because the number of subjects included in this group was too small to conduct appropriate analyses (n=13).

Statistical significance was assumed at a *P* value <0.05 and for interactions at a *P* value <0.10. Statistical analyses were performed using SAS 9.4 (SAS Institute, Inc, Cary, NC).

Results

General Characteristics of the Study Population

Plasma chemerin levels ranged from 32.1 to 193.0 ng/mL with a mean level of 97.8 ng/mL. In 1620 subjects (40.5%) of our study population, a carotid plaque was observed. With 1.2% (n=47), the prevalence of carotid stenosis was lower. The values of the cIMT ranged from 0.32 to 1.37 mm with an average value of 0.59 mm. ABI was measured in a subpopulation of 2488 subjects and showed a range of 0.54 to 1.70 with a mean of 1.13. In Table 1, further descriptive statistics of the study population stratified by tertiles of plasma chemerin levels are presented. In comparison with subjects with low plasma chemerin levels, those with high chemerin levels were more often women, were older, had higher BMI, and had an unfavorable metabolic and inflammatory profile. Furthermore, these subjects had more often carotid plaque and carotid stenosis, higher cIMT values, and lower ABI than subjects with low plasma chemerin levels (Table 1). The sex- and age-specific cutoff values for a high cIMT and a low ABI are presented in Table 2. In subjects showing evidence for a subclinical atherosclerosis (defined via the presence of carotid plaque, carotid stenosis, high cIMT, or low ABI), plasma chemerin levels were significantly higher than in healthy people (Figure 1).

Associations of Plasma Chemerin With Subclinical Parameters of Atherosclerosis

ANOVA models adjusted for age and sex revealed positive associations of plasma chemerin levels with cIMT and inverse associations of plasma chemerin with ABI (Figure 2). After further adjustment for waist circumference, other metabolic and inflammatory parameters, as well as for systolic blood pressure and renal function, only the inverse association between chemerin and ABI remained significant. In detail, mean ABI values in the highest chemerin quartile are ≈0.03 U (2.7%) lower than those in the lowest chemerin quartile. Multivariable linear regression models confirmed the inverse association of plasma chemerin with ABI (Figure 2; Table 3).

After adjustment for age and sex, logistic regression models revealed positive associations between plasma chemerin and the presence of carotid plaque, carotid stenosis, or a high cIMT, but all these associations were not significant anymore after adjustment for waist circumference, other metabolic and inflammatory parameters, as well as systolic blood pressure and renal function (Table 4). In contrast, the association between plasma chemerin and a low ABI remained consistent even in the fully adjusted model, and interestingly, the strength of the association even increased. Specifically, each increase in plasma chemerin per 25 ng/mL was associated with a 30% higher odd of having an ABI value below the 25th age- and sex-specific percentile (Table 4).

The exclusion of subjects with a known history of cardiovascular diseases did not change the results (Tables 3 and 4). Similarly, the results stayed consistent when analyzing only subjects with ABI values ≤1.3 (data not shown).

Table 1. Descriptive Statistics of the Study Population Stratified by Tertiles of Plasma Chemerin Concentrations

	First Tertile	Second Tertile	Third Tertile	
	<88.17 ng/mL	88.17–110.02 ng/mL	≥110.03 ng/mL	P Value
Men, %	55.7	48.9	41.5	<0.01
Age, y	45 (35–56)	53 (41–64)	59 (48–69)	<0.01
Smoking, %				<0.01
Never smokers	33.9	34.2	40.9	
Former smokers	36.2	39.0	35.6	
Current smokers	29.9	26.8	23.5	
BMI, kg/m²	25.4 (22.7–28.2)	27.8 (24.8–30.8)	29.7 (26.7–33.4)	<0.01
Waist circumference, cm	84.1 (75.4–93.9)	90.6 (80.8–101.0)	96.4 (88.0–105.2)	<0.01
HbA1c, %	5.1 (4.7–5.4)	5.2 (4.9–5.6)	5.5 (5.1–5.9)	<0.01
Total triglycerides, mmol/L	1.1 (0.8–1.6)	1.4 (1.0–2.0)	1.7 (1.2–2.4)	<0.01
Total cholesterol, mmol/L	5.2 (4.5–5.9)	5.4 (4.7–6.2)	5.6 (4.9–6.3)	<0.01
LDL cholesterol, mmol/L	3.1 (2.5–3.7)	3.3 (2.7–4.0)	3.5 (2.9–4.1)	<0.01
HDL cholesterol, mmol/L	1.5 (1.2–1.7)	1.4 (1.2–1.7)	1.3 (1.1–1.6)	<0.01
Systolic BP, mm Hg	122.8 (111.5–135.5)	127.5 (115.0–139.0)	129.5 (118.5–142.5)	<0.01
Diastolic BP, mm Hg	75.0 (69.0–82.5)	77.0 (71.0–84.0)	77.5 (71.0–84.0)	<0.01
hsCRP, mg/L	0.8 (0.4–1.4)	1.3 (0.7–2.5)	2.4 (1.2–4.8)	<0.01
eGFR, mL/min per 1.73 m²	92.6 (82.6–107.8)	87.1 (76.3–100.4)	78.8 (66.8–91.3)	<0.01
Carotid plaque, %	27.6	41.6	52.1	<0.01
Carotid artery stenosis, %	0.6	0.8	2.1	<0.01
cIMT, mm	0.54 (0.47–0.64)	0.59 (0.51–0.71)	0.63 (0.54–0.76)	<0.01
ABI*	1.13 (1.07–1.18)	1.13 (1.08–1.19)	1.12 (1.07–1.18)	0.04
Chemerin, ng/mL	76.2 (68.0–82.5)	97.8 (92.9–103.6)	126.5 (117.0–141.1)	<0.01

Continuous data are presented as median (25th–75th quartile); nominal data are given as percentages. χ² test (nominal data) or Mann-Whitney U test (continuous data) was used for comparisons between chemerin groups. Carotid plaque was defined if at least 1 carotid arterial segment was affected by plaque. Carotid stenosis was defined as a stenosis of >20% according to NASCET criteria. ABI indicates ankle-brachial index; BMI, body mass index; BP, blood pressure; cIMT, carotid intima-media thickness; eGFR, estimated glomerular filtration rate; HbA1c, glycohemoglobin; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; and NASCET, North American Symptomatic Endarterectomy Trial.

*Measurements of the ABI were available in a subpopulation of 2488 subjects.

Additional adjustment for lipid-lowering and antihypertensive medication in the regression models did not influence the presented results notably (Tables I and II in the online-only Data Supplement). Furthermore, the repetition of analyses in subsamples of BMI groups has shown that the observed inverse association between plasma chemerin and ABI can be equally detected in normal-weight, overweight, and obese subjects (Tables III and IV in the online-only Data Supplement).

Discussion

The present study used for the first time data form a general population to examine the associations of plasma chemerin levels with subclinical parameters of atherosclerosis. Linear and logistic regression analyses adjusted for age and sex revealed that high plasma chemerin levels are significantly associated with a higher burden of subclinical atherosclerosis, represented by lower ABI, higher cIMT, or the presence of carotid plaque or carotid stenosis. After additional adjustment for waist circumference, other metabolic and inflammatory

parameters, as well as systolic blood pressure and renal function, the observed associations between chemerin and high cIMT, the presence of carotid plaque or carotid stenosis missed statistical significance, suggesting that these parameters act as confounding variables on the association. In contrast, the

Table 2. Sex- and Age-Specific Cutoff Values for High cIMT and Low ABI

	75th Quartile cIMT, mm		25th Quartile ABI	
Age, y	Men	Women	Men	Women
20–29	0.49	0.46	1.04	1.04
30–39	0.55	0.51	1.04	1.05
40–49	0.64	0.58	1.07	1.08
50–59	0.72	0.67	1.08	1.08
60–69	0.81	0.75	1.08	1.08
70+	0.87	0.86	1.07	1.07

High cIMT was defined as cIMT >75th sex- and age-specific quartile (n=997). Low ABI was defined as ABI <25th sex- and age-specific quartile (n=579). ABI indicates ankle-brachial index; and cIMT, carotid intima-media thickness.
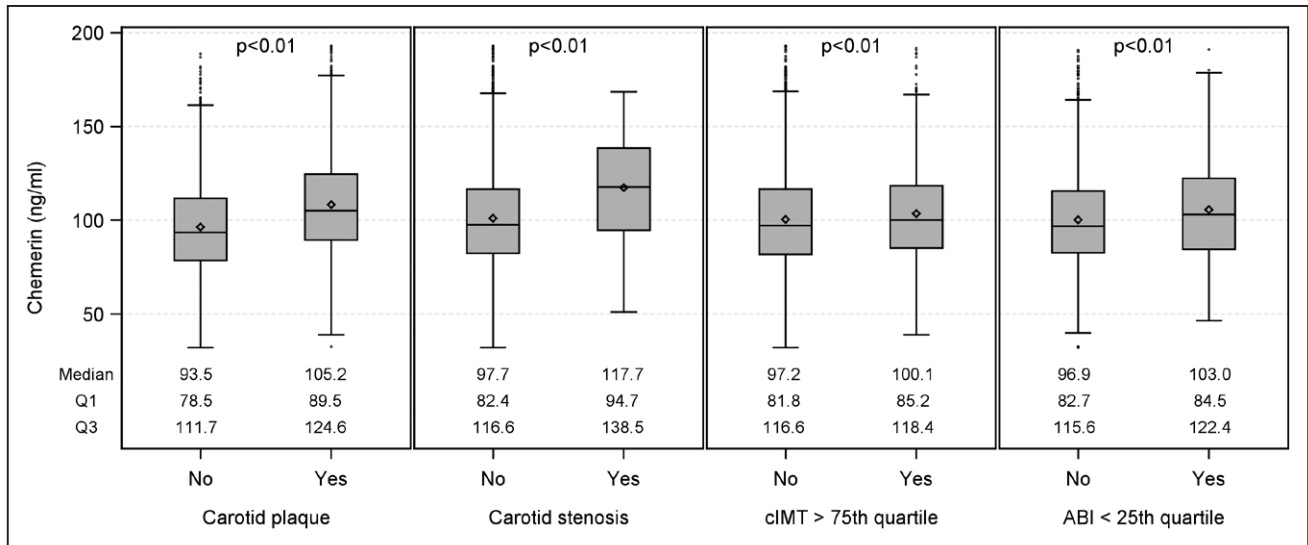
Figure 1. Box plots of plasma chemerin concentrations in subjects with and without evidence for subclinical atherosclerosis. Exact values for median, 25th (Q1), and 75th quartile (Q3) are given. Mann-Whitney *U* test was used for comparisons. Carotid plaque was defined if at least 1 carotid arterial segment was affected by plaque. Carotid stenosis was defined as a stenosis of >20% according to NASCET (North American Symptomatic Endarterectomy Trial) criteria. High carotid intima-media thickness (cIMT) was defined as cIMT >75th sex- and age-specific quartile. Low ankle-brachial index (ABI) was defined as ABI <25th sex- and age-specific quartile.

inverse association of chemerin with ABI remained statistically significant after adjustment for the same set of variables, and the strength of the association even increased. We assume that the latter can be explained by the fact that the additional variables included in model 2 explain a great part of the unexplained variance between chemerin and ABI, which was seen in the first model. This in turn has the consequence that even small effects of the exposure variable become visible.

During the last years, an increasing number of clinical studies have investigated the relation of circulating chemerin levels with atherosclerosis. In line with our observations, a recent study that analyzed data from diabetic, prediabetic, and healthy patients has observed no association of serum chemerin with cIMT when using adjusted regression models,[25] whereas 2 further cross-sectional reports have detected a significant positive association between these parameters.[18,19] To date, the relation between circulating chemerin and ABI has been examined only in 2 clinical studies,[19,26] but in contrast to our results, none of them has detected significant associations. Studies that used carotid plaques or carotid stenosis as an outcome variable revealed conflicting results by showing positive,[12,14,15] inverse,[22] or insignificant[21,23] relations. The possible reasons for these large discrepancies can be manifold. Differences in the characteristics and ethnicity of the study populations, in the used ELISA kits to measure circulating chemerin levels, in the parameters for assessment of subclinical atherosclerosis, or in the considered adjustment set should be mentioned in this respect. cIMT, stenotic or nonstenotic plaques of the carotid bifurcation, and ABI are biologically and genetically distinct markers of atherosclerosis.[35] The cIMT represents adaptive changes of the intimal and medial layers of the arterial wall to the exposure of vascular risk factors, whereas both carotid plaques and ABI express apparent atherosclerotic disease of the affected arteries. Moreover, carotid plaques and ABI differ with respect

to the affected arterial territory and the severity of atherosclerotic disease.[35,36] Consequently, we found no significant correlation between cIMT and ABI after adjustment for age and sex (partially adjusted Pearson correlation coefficient: $r=-0.003$; $P=0.89$).
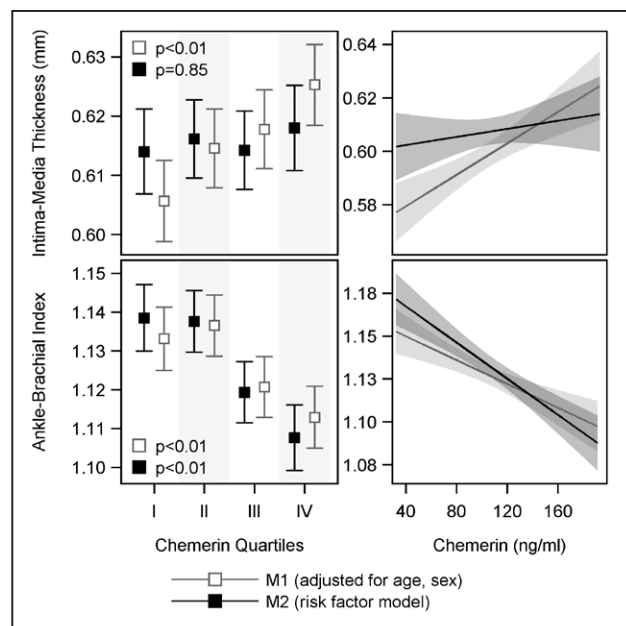

Figure 2. Associations of plasma chemerin with carotid intima-media thickness and ankle-brachial index (ABI). **Left,** Adjusted means with 95% confidence intervals (CI) according to sex-specific quartiles of chemerin calculated by ANOVA; **right,** estimated linear regression lines with corresponding 95% CI. Model 1: adjusted for age and sex (M1, white box, grey line); model 2: additionally adjusted for waist circumference, glycohemoglobin, systolic blood pressure, low-density lipoprotein cholesterol, high-sensitivity C-reactive protein, and estimated glomerular filtration rate (M2, black box, black line). Inverse probability weighting was applied for ABI to account for missing data.

Table 3. Results From Linear Regression Analysis: Association of Plasma Chemerin (as Continuous Variable) With cIMT and ABI

	n	Model 1*			Model 2*		
		β per 25 ng/mL Increase in Chemerin	SE	P Value	β per 25 ng/mL Increase in Chemerin	SE	P Value
cIMT							
Whole	4003	0.0073	0.0017	<0.01	0.0019	0.0020	0.34
Subpop1	3414	0.0056	0.0018	<0.01	−0.0009	0.0020	0.67
ABI†							
Whole	2488	−0.0087	0.0020	<0.01	−0.0132	0.0023	<0.01
Subpop1	2132	−0.0080	0.0021	<0.01	−0.0121	0.0024	<0.01

ABI indicates ankle-brachial index; cIMT, carotid intima-media thickness; HbA1c, glycohemoglobin; hsCRP, high-sensitivity C-reactive protein; and LDL, low-density lipoprotein.

*Model 1: adjusted for age, sex; model 2: additionally adjusted for waist circumference, HbA1c, systolic blood pressure, LDL cholesterol, hsCRP, and estimated glomerular filtration rate.

†Inverse probability weighting was applied for ABI to account for missing data. Analyses were done using whole population sample (whole) and repeated in a subsample of subjects without known clinical history of cardiovascular diseases (subpop1).

Several population-based cohort studies have reported a significant u-shaped relation between baseline ABI and the risk of future cardiovascular events.[37,38] Consequently, ABI is considered not only as a clinical tool to quantify the severity of peripheral artery disease but also as an important predictive marker for cardiovascular risk.[39,40] Meanwhile, it is commonly accepted that an ABI ≤0.9 or >1.4 identifies subjects with a high risk for cardiovascular events.[31] Some studies have even indicated that

an ABI <1.10 is related to a higher risk profile than an ABI ≥1.10 and <1.4.[37,40,41] However, most existing studies that analyzed the relation between baseline ABI levels and future cardiovascular risk are based on data from the elderly population (baseline age, 45+ years).[37,42–44] As far as we know, only 1 of the existing population-based studies included also younger subjects.[40] Similar to the studies in elderly subjects, this study[40] found that each 0.1 U decrease in ABI is associated with an increase in the

Table 4. Results From Logistic Regression Analysis: Association of Plasma Chemerin (as Continuous Variable) With the Odds for Carotid Plaque, Carotid Stenosis, High cIMT, or Low ABI

Outcome	n	Model 1*		Model 2*	
		OR (95% CI) per 25 ng/mL Increase in Chemerin	P Value	OR (95% CI) per 25 ng/mL Increase in Chemerin	P Value
Plaque					
Whole	1620/4003	1.10 (1.01–1.20)	0.03	1.06 (0.97–1.17)	0.21
Subpop1	1233/3414	1.08 (0.99–1.19)	0.10	1.03 (0.93–1.14)	0.60
Stenosis					
Whole	47/4003	1.36 (1.04–1.78)	0.02	1.39 (1.03–1.88)	0.03
Subpop1	27/3414	1.16 (0.81–1.67)	0.43	1.29 (0.86–1.93)	0.21
High cIMT					
Whole	997/4003	1.11 (1.03–1.19)	<0.01	0.99 (0.91–1.08)	0.88
Subpop1	832/3414	1.09 (1.00–1.18)	0.04	0.96 (0.87–1.05)	0.37
Low ABI†					
Whole	579/2488	1.23 (1.14–1.32)	<0.01	1.30 (1.20–1.41)	<0.01
Subpop1	484/2132	1.22 (1.13–1.32)	<0.01	1.29 (1.18–1.42)	<0.01

ABI indicates ankle-brachial index; CI, confidence interval; cIMT, carotid intima-media thickness; HbA1c, glycohemoglobin; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NASCET, North American Symptomatic Endarterectomy Trial; and OR, odds ratio.

*Model 1: adjusted for age, sex; model 2: additionally adjusted for waist circumference, HbA1c, systolic blood pressure, LDL cholesterol, hsCRP, and estimated glomerular filtration rate. Carotid plaque was defined if at least 1 carotid arterial segment was affected by plaque. Carotid stenosis was defined as a stenosis of >20% according to NASCET criteria. High cIMT was defined as cIMT >75th sex- and age-specific quartile. Low ABI was defined as ABI <25th sex- and age-specific quartile.

†Inverse probability weighting was applied for ABI to account for missing data. Analyses were done using the whole population sample (whole) and repeated in a subsample of subjects without known clinical history of cardiovascular diseases (subpop1).

cardiovascular and total mortality of ≈25% (baseline age range, 25–74 years; follow-up period, 13 years).[40] Nevertheless, the meaning of ABI as predictor of future cardiovascular events in a young and comparable healthy population as ours is still not clear. Thus, it remains uncertain whether a low ABI as assessed in this population really represents a reliable proxy for peripheral artery diseases and, therefore, predicts future cardiovascular events. However, the observed inverse association between chemerin and ABI remained significant when we restricted our analyses to subjects <45 years (data not shown). Nevertheless, longitudinal analyses are urgently needed to clarify whether high chemerin levels are truly predictive for future peripheral artery disease or other cardiovascular events.

In general, the development of atherosclerotic lesions is a dynamic and complex process that is still not fully understood. During the previous decades, adipokines, such as chemerin, have been identified as critical mediators within this process.[2] Existing preclinical and animal studies reveal that chemerin with its large functional scope might have various actions in the vascular system.[8–11,45–48]

Atherosclerotic lesions develop in blood vessels that are enclosed by a layer of perivascular adipose tissue, which in turn is known to secrete a multitude of adipokines.[49] Because of the immediate proximity of the perivascular adipose tissue, it is likely that these adipokines enter into the vascular wall where they further interact with cells from the different artery layers. Chemerin and its receptor CMKLR1 have been identified to be produced by perivascular and epicardial adipose tissue.[50,51] Furthermore, a high chemerin expression profile in these tissues has been associated with aortic and coronary atherosclerosis.[50,51] Hence, chemerin as an adipokine that is secreted from inflamed perivascular adipose tissue might influence the atherosclerotic process.

The thin layer of endothelial cells that lines the interior of the blood vessels plays an essential role in the atherosclerotic process.[52] Chemerin and its receptor CMKLR1 both have been observed to be expressed in human endothelial cells,[8] and evidence is given that chemerin might impair vascular relaxation by reducing the NO production in these cells.[9] A reduced NO production is known to increase endothelial expression of vascular adhesion molecules and to promote leukocyte adhesion to the vessel wall.[52] Both processes have already been linked to chemerin by showing that chemerin induces the expression of adhesion molecule 1 and E-selectin in endothelial cells[45] and promotes leukocyte recruitment.[10] Together, these studies suggest that chemerin might induce endothelial dysfunction—an important pathogenic mechanism that initiates the atherosclerotic process.

By inducing angiogenesis in endothelial cells, chemerin seems to have also a function as a growth factor. Experimental studies have demonstrated that chemerin significantly stimulated the formation of new blood vessels in endothelial cells through activation of MMPs (matrix metalloproteinases) 2 and 9.[8,11] The observed chemerin-driven angiogenesis might be a result of increased hypoxia that again has been observed to enhance chemerin expression in endothelial cells.[48] The role of angiogenesis in atherosclerosis is a highly contentious issue.[53] However, a variety of studies have indicated that angiogenesis contributes to plaque growth, instability, and

rupture[54] possibly because of the fact that the newly formed vessels are more permeable.[55] Further studies are needed to identify the consequences of chemerin-induced angiogenesis during the atherosclerotic process.

As a chemokine, chemerin promotes the recruitment of macrophages and dendritic cells to migrate to sites of tissue injury.[5] Macrophages form foam cells, and these cells again secrete a variety of inflammatory mediators that induce vascular smooth muscle cell migration and proliferation.[53] It has been observed that chemerin induces vascular smooth muscle cell proliferation and migration by increasing the production of reactive oxygen species in these cells and phosphorylation of Akt and ERK (extracellular signal-regulated kinase).[46,47] Correspondingly, high chemerin and CMKLR1 expression levels have been found in foam cells and vascular smooth muscle cells, and these expression levels significantly correlated with the severity of the atherosclerotic lesion.[50,51,56] In summary, the presented studies reveal that chemerin might be a critical mediator in several inflammatory stages during the atherosclerotic process.

Strengths and Limitations

To the best of our knowledge, this is the most comprehensive epidemiological analysis of associations between circulating chemerin levels and subclinical atherosclerotic parameters to date. Data from a large population-based study was used, which ensures a highly standardized data collection and accurate assessment of subclinical cardiovascular outcomes. Potential limitations to the observed associations and to the generalizability of our findings arise especially from the cross-sectional design and the given age range of the present study. Therefore, causal relations between the parameters of interest could not be investigated, and the prevalence of stenosis in the given population sample was quite too small to achieve sufficient statistical power. Furthermore, only few cases (n=27) had an ABI below the commonly used threshold of 0.9 what forced us to use a quite unusual definition of a low ABI, as an ABI value below the 25th sex- and age-specific quartile.

Conclusions

The present study revealed a modest but significant inverse association between plasma chemerin levels and ABI that remained consistent after adjustment for waist circumference, other metabolic and inflammatory parameters, as well as systolic blood pressure and renal function. However, the association of plasma chemerin with cIMT, carotid plaque, or carotid stenosis was not significant after adjustment for the same set of confounders. Our results suggest that chemerin might have different associations depending on the stage and region of the atherosclerotic lesion. We assume that the observed inverse association between plasma chemerin and ABI indicates a link between high circulating chemerin levels and a future presence of peripheral arterial disease. But further longitudinal analyses are urgently needed to validate the role of circulating chemerin in the vascular system.

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Disclosures

None.

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Highlights

- This study assessed for the first time the association of plasma chemerin levels with parameters of subclinical atherosclerosis in a population-based setting.
- Plasma chemerin levels were measured in a unique sample of >4000 subjects, and its association with different subclinical atherosclerotic parameters was investigated.
- After adjustment for waist circumference, metabolic and inflammatory parameters, as well as systolic blood pressure and renal function, a modest inverse association of plasma chemerin with ankle-brachial index but not with carotid intima-media thickness, carotid plaque, or carotid stenosis was observed.
- Because the investigated subclinical atherosclerotic parameters are representative for the atherosclerotic burden of different arterial regions and disease stages, we conclude that the value of chemerin as a marker of higher atherosclerotic risk differs depending on the affected arterial region and on the disease stage.