Circulating Concentrations of the Novel Adipokine Chemerin Are Associated with Cardiovascular Disease Risk in Rheumatoid Arthritis

Patrick H. Dessein, Linda Tsang, Angela J. Woodiwiss, Gavin R. Norton, and Ahmed Solomon

ABSTRACT. ***Objective.*** Depending on physiological context, the adipokine chemerin can reduce or enhance cardiovascular risk. We investigated whether chemerin concentrations represent cardiovascular disease risk in rheumatoid arthritis (RA).

Methods. We assessed ELISA-determined chemerin concentrations and those of 4 early endothelial activation molecules as well as angiopoietin 2, which mediates angiogenesis and thereby contributes to advanced atherosclerosis, the common carotid artery intima-media thickness (cIMT), and carotid artery plaque by ultrasound in 236 patients (114 black and 122 white) with RA. Relationships were identified in potential confounder and mediator-adjusted mixed regression models.

Results. Mean (SD) chemerin and median (interquartile range) angiopoietin 2 concentrations were 114 (35) ng/ml and 2560 (2044–3341) pg/ml, respectively; the mean (SD) cIMT was 0.708 (0.110) mm, and 40.3% of patients had plaque. Chemerin concentrations were not related to those of early endothelial activation molecules, but associated with those of angiopoietin 2 [β SE = 0.002 (0.0004), $p < 0.0001$] and plaque [OR 1.006 (95% CI 1.00–1.013), $p = 0.05$] in all patients. The presence of major conventional cardiovascular risk factors, generalized and abdominal obesity, and RA severity markers modified the independent chemerin-cardiovascular risk relations (interaction $p < 0.05$). Consequently, chemerin concentrations were associated with cIMT in those with but not without overweight or generalized obesity and abdominal obesity [β SE = 0.001 (0.0003), $p = 0.005$ and 0.001 (0.0001), $p = 0.001$ vs –0.001 (0.0004), $p = 0.2$ and –0.0002 (0.0004), $p = 0.6$, respectively], and with plaque in those without but not with generalized obesity [OR 1.008 (95% CI) 1.000–1.016, $p = 0.03$ vs 1.003 (0.990–1.017), $p = 0.6$, respectively]. The β (SE) for the chemerin-intima-media thickness relations in patients with overweight or generalized obesity and abdominal obesity were larger than in those without these characteristics ($p < 0.0001$ and = 0.04, respectively).

Conclusion. Chemerin is associated with endothelial activation and atherosclerosis in RA. Adiposity influences the chemerin-atherosclerotic phenotype relations in RA. (J Rheumatol First Release July 15 2014; doi:10.3899/jrheum.140122)

Key Indexing Terms:

RHEUMATOID ARTHRITIS	CARDIOVASCULAR RISK	OBESITY
ENDOTHELIAL ACTIVATION	ATHEROSCLEROSIS	CHEMERIN

Rheumatoid arthritis (RA) predisposes substantially to traditional and nontraditional risk factor dependent atherosclerotic cardiovascular disease (CVD) with a prevalence and incidence as large as in diabetes[1,2,3]. Atherogenesis in RA remains poorly understood, and currently recommended cardiovascular risk strategies[4] have potentially important shortcomings[5,6]. It is against this background that the need for identifying novel biomarkers of cardiovascular risk in this inflammatory disease has been emphasized[5,6].

Chemerin, also known as tazarotene-induced gene 2 or retinoic acid receptor responder 2, was originally identified as a gene upregulated in psoriatic skin by the synthetic retinoid tazarotene in 1997[7]. In 2007, chemerin was identified as a novel adipokine that regulates adipogenesis and adipocyte metabolism[7]. Chemerin is a natural ligand and chemotactic signal for cells expressing G protein-coupled receptor chemokine-like receptor 1[7,8].

A large body of evidence derived from mechanistic and clinical studies indicates that chemerin is proinflammatory and implicated in metabolic risk[7,8]. As an early biomarker of metabolic and inflammatory disease, chemerin concentrations predicted incident metabolic risk more strongly than

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measures of insulin resistance and leptin concentrations in a population without clinical symptoms of metabolic syndrome[8]. Chemerin also induces the production of E-selectin and intercellular adhesion molecule 1 (ICAM-1) in endothelial cells[9], and further increases angiogenesis[10], which contributes to advanced atherosclerosis by augmenting plaque development and destabilization[11]. Importantly, in the present context, chemerin participates in normal physiological processes and also exhibits protective properties in that, depending on (patho)physiological status, it can have antiinflammatory activity, and both reduced and elevated chemerin signaling may cause aberrant glucose metabolism[8]. Therefore, disease status and severity require consideration upon evaluating the biological effects of chemerin[8].

As applies to other adipokines[12], chemerin is involved in the pathophysiology of RA[13,14,15]. Chemerin is expressed in synovial endothelial, lining, and sublining cells, and its production is upregulated by tumor necrosis factor-α (TNF-α) and interferon-γ in RA; chemerin stimulated the production of interleukin 6 (IL-6), chemokine (C-C motif) ligand 2, Toll-like receptor 4 mRNA, and matrix metalloproteinase 3 by fibroblast-like synoviocytes[13,14].

Adipokines other than chemerin are linked to metabolic risk, early endothelial activation, and inflammation in RA[16,17,18,19,20,21,22,23,24]. However, circulating concentrations of these molecules were reported to be unrelated to atherosclerosis[25], except for retinol-binding protein 4[26]. In our study, we examined the independent relationships between chemerin concentrations and metabolic risk, surrogate markers of early endothelial activation as well as angiogenesis, and atherosclerosis in 236 African (114 black and 122 white) patients with RA. We also determined whether the presence of major traditional risk factors, excess adiposity, and RA severity markers modify these relationships.

MATERIALS AND METHODS

Patients. We conducted our study according to the principles outlined in the Helsinki declaration. The Human Research Ethics Committee (Medical) from the University of the Witwatersrand in Johannesburg, South Africa, approved the protocol (approval number: M06-07-33). Participants gave informed, written consent. This investigation forms part of an ongoing study on cardiovascular risk in RA[18,19,21,22]. There were 236 consecutive African patients (114 black and 122 white) enrolled who met the 1988 American College of Rheumatology (ACR) and 2010 ACR/European League Against Rheumatism criteria for RA[27,28]. All invited participants agreed to participate. Data were missing in fewer than 5% of any of the recorded characteristics.

Assessments. Baseline characteristics and conventional metabolic risk factors were recorded using previously reported methods[18,19,21,22]. Briefly, we recorded demographic features and lifestyle factors. Height, weight, and waist and hip circumference were measured using standard approaches. The body mass index (BMI) was calculated, and abdominal obesity and fat distribution were estimated by waist circumference and waist circumference-hip ratio, respectively. We recorded disease duration and rheumatoid factor (RF) status. Disease activity was assessed by the Clinical Disease Activity Index and the Disease Activity Score in 28 joints (DAS28). Extraarticular manifestations included the current or previously recorded (hospital record review) presence of pericarditis, pleuritis, Felty's syndrome, cutaneous vasculitis, neuropathy, scleritis or episcleritis, retinal vasculitis, glomerulonephritis, vasculitis affecting other organs, amyloidosis, keratoconjunctivitis sicca, xerostomia, Sjogren syndrome, pulmonary fibrosis, bronchiolitis obliterans organizing pneumonia, cervical myelopathy, subcutaneous nodules, and rheumatoid nodules in other locations. C-reactive protein (CRP) concentrations were determined using immunoturbidimetric methods, and those of IL-6 by ELISA. Standard laboratory blood tests of erythrocyte sedimentation rate, renal and liver function, hematological variables, lipids, and glucose were performed. The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease equation. Cardiovascular drug use was recorded.

Recorded metabolic risk factors included systolic, diastolic, and mean blood pressure, lipid concentrations and ratios, and glucose levels. Hypertension was defined as an average systolic blood pressure ≥ 140 or/and diastolic blood pressure ≥ 90 mmHg or/and current use of antihypertensive medications. Dyslipidemia was diagnosed when the atherogenic index, i.e., the cholesterol/high-density lipoprotein (HDL) cholesterol ratio was > 4[4]. Diabetes was identified as the use of glucose lowering agents or a fasting plasma glucose ≥ 7 mmol/l.

We evaluated concentrations of 5 other adipokines, including total and high molecular weight adiponectin, leptin, and resistin as previously reported[18,19,21,22], as well as retinol-binding protein 4 using a solid-phase sandwich ELISA (QuantikineHS, R&D Systems Inc.) with a lower detection limit of 0.224 μg/ml and the interassay and intraassay coefficients of variation of 7.2% and 6.9%, respectively.

We measured early endothelial activation molecule concentrations, including those of soluble E-selectin, vascular cell adhesion molecule 1 (VCAM-1), ICAM-1, and monocyte chemoattractant protein 1 (MCP-1), as well as angiopoietin 2, and using solid-phase sandwich ELISA (QuantikineHS). Their lower detection limits were 0.009 ng/l, 0.6 ng/l, 0.096 ng/l, 5.0 pg/ml, and 1.2 pg/ml, respectively; their interassay and intraassay coefficients of variation were 7.9 and 5.8, 7.0 and 3.1, 5.5 and 4.6, 5.7 and 5.8, and 8.9 and 5.9%, respectively.

Carotid artery ultrasound (US) measurements were done by 2 operators, 1 on private healthcare patients and 1 on public healthcare patients. Both resulted in images of at least 1-cm length of the distal common carotid arteries for measurement of the intima-media thickness of the far wall from an optimal angle of incidence defined as the longitudinal angle of approach where both branches of the internal and external carotid artery are visualized simultaneously[29], and with high-resolution B-mode US (Image Point, Hewlett Packard and SonoCalc IMT, Sonosite Inc.) using linear array 7.5 MHz probes. The details of the methodology were reported[30]. The equipment used with the public healthcare patients involved the application of a unique semiautomated border detection program that was previously found to provide highly reproducible results[29]. The intima-media thicknesses in the left and right common carotid artery were measured, and the carotid artery intima-media thickness (cIMT) was defined as the mean of these. Carotid artery plaque was defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding intima-media thickness value, or demonstrates a thickness of > 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface[31]. Both operators were blinded to the cardiovascular risk profiles of the patients. Repeat US examinations by both operators on 23 patients revealed intraobserver coefficients of variation of 5.8% and 4.1% for private healthcare patients and public healthcare patients, respectively, and an interobserver coefficient of variation of 8.0% for measurements made by the 2 operators. Both operators identified carotid artery bulb or/and internal carotid artery plaque in 11 of these 23 patients, with full agreement.

Chemerin concentrations were quantified using a solid-phase sandwich ELISA (QuantikineHS). The lower detection limit was 1.08 pg/ml and the interassay and intraassay coefficients of variation were 7.3% and 3.9%, respectively.

Data management analysis. Dichotomous variables are expressed as proportions or percentages, and continuous variables as mean (SD) or median (interquartile range) when non-normally distributed. Non-normally distributed characteristics were also logarithmically transformed prior to their inclusion in multivariable statistical analysis. An endothelial activation score was used to provide a summary measure of early endothelial activation and was calculated from SD (z) scores as follows: [z (selectin) + z (VCAM-1) + z (ICAM-1) + z (MCP-1)][19,22].

Associations of age at disease onset or at the time of the study, and sex and population grouping with chemerin concentrations were assessed by entering the respective characteristics together in single mixed regression models. Associations of other baseline characteristics with chemerin concentrations were evaluated in models with adjustment for demographic characteristics that included age at the time of the study and not age at disease onset, because the latter was not related to chemerin concentrations.

The independent relations of chemerin concentrations with metabolic risk factors were assessed in demographic characteristic, waist circumference, GFR, leflunomide (LEF) and TNF-α blockade use (potential confounders or/and determinants identified in previous analysis), and cardiovascular drug use adjusted mixed linear regression models. The independent relations of chemerin concentrations with endothelial activation, cIMT, and plaque were determined in Framingham score (calculated from age, sex, and major conventional risk factors), race, waist, GFR, CRP, and leptin concentrations, and LEF and TNF-α blockade use adjusted mixed (linear or logistic as appropriate) regression models.

Patients with RA who experience conventional risk factors or severe disease are reportedly at high risk of CVD[1,2,4]. For these reasons, together with the dependence of chemerin effects on physiological context[8] and our recent experience with adipokine metabolism in RA[18,19,20,21,22], we assessed the effect of patient characteristics on chemerin-cardiovascular risk relations by adding interaction terms to the models, and stratified analysis when indicated (significant interaction p values), that is, in subgroups with and without patient characteristics of interest in the present context. For this purpose, patients with a BMI of ≥ 30 kg/m^2 and those who met the National Cholesterol Education Program for metabolic syndrome waist criterion[32] were considered to sustain overall and abdominal obesity, respectively. When appropriate, patients were categorized in subgroups based on median values. In view of the small number of patients who were RF-positive or had extraarticular features, sensitivity analysis in subgroups based on the presence or absence of these characteristics was not performed.

Statistical computations were made using the GB Stat program (Dynamic Microsystems Inc.) and SAS software, version 9.1 (The SAS Institute). Significance was set at $p \leq 0.05$.

RESULTS

Descriptive statistics of the recorded characteristics are given in Table 1. The mean (SD) Framingham score was 5 (7), as determined using an algorithm[33]. However, this characteristic was non-normally distributed with a median (interquartile range) value of only 2 (1–6). All patients were using synthetic disease-modifying agents and only 1 of them used a biologic agent other than TNF-α blockade (rituximab).

Associations of baseline characteristics with chemerin concentrations. As presented in Table 2, demographic characteristics, anthropometric measures, the use of antihypertensives, disease activity, and the GFR were each significantly associated with chemerin concentrations. Among the disease activity variables, CRP concentrations were most strongly related to chemerin concentrations. LEF and TNF-α blockade use were also borderline related to chemerin concentrations (p = 0.06 and 0.1, respectively), and hence additionally included as potential confounders in subsequent analyses. In separate analysis, when BMI and waist were entered into the same demographic characteristic and GFR-adjusted model, only waist circumference remained associated with chemerin concentrations, the DAS28 and waist were related to chemerin concentrations independent of one another, and the antihypertensive agent use-chemerin concentrations relation was explained by abdominal adiposity (data not shown). In view of these results, waist circumference was the anthropometric measure that was included in subsequent models. All cardiovascular drug use was consistently forced into the models in which chemerin-cardiovascular risk relations were evaluated.

Independent relationships of chemerin concentrations with metabolic risk. As also shown in Table 2, in all 236 patients, chemerin concentrations were independently related to those of leptin. Chemerin levels were not related to any of the other metabolic risk factors.

An RA duration of > 10 years affected the chemerin-HDL cholesterol concentration and chemerin-cholesterol-HDL cholesterol ratio associations (interaction p = 0.04 and 0.006, respectively). The presence of deformed joints affected the chemerin-systolic, -diastolic, and -mean blood pressure relations (interaction p = 0.01, 0.03, and 0.01, respectively). In stratified analysis, chemerin concentrations were independently associated with systolic blood pressure, diastolic blood pressure, mean blood pressure, and cholesterol-HDL-cholesterol ratio in patients with but not in those without a disease duration of > 10 years [β (SE) = 0.117 (0.056), p = 0.03, 0.068 (0.033), p = 0.04, 0.100 (0.045), p = 0.03 and 0.006 (0.005), p = 0.03 vs –0.031 (0.078), p = 0.7, –0.050 (0.043), p = 0.2, –0.038 (0.062), p = 0.5 and 0.001 (0.004), p = 0.7, respectively], and with systolic and diastolic blood pressure in patients with but not in those without deformed joints [β (SE) = 0.111 (0.056), p = 0.05 and 0.090 (0.046), p = 0.05 vs –0.089 (0.071), p = 0.2 and –0.072 (0.057), p = 0.2, respectively].

Generalized obesity affected the chemerin-leptin and chemerin-retinol binding protein 4 concentration relations (interaction p = 0.02 and 0.03, respectively); abdominal obesity affected on the chemerin-total adiponectin concentration association (interaction p = 0.03). In stratified analysis, chemerin concentrations were related to leptin levels in patients without but not in those with generalized and abdominal obesity [β (SE) = 0.003 (0.001), p = 0.0008 and 0.003 (0.001), p = 0.02 vs –0.001 (0.001), p = 0.5 and 0.001 (0.001), p = 0.2, respectively].

Independent relationships of chemerin concentrations with endothelial activation and atherosclerosis. Table 3 shows that whereas chemerin concentrations were not related to early endothelial activation in all patients, they were

Table 1. Recorded characteristics in 236 patients with RA. Dichotomous variables are expressed as proportions or percentages and continuous characteristics as mean ± SD or median (interquartile range).

Demographic characteristics	
Age at disease onset, yrs	43.5 (13.0)
Age at study time, yrs	57.1 (10.8)
Female sex	83.5
Black	48.3
White	51.7
Lifestyle factors	
Exercise	36.8
Alcohol use	20.9
Current smoking	6.8
Anthropometry	
Body mass index, kg/m^2	27.5 (6.0)
Waist circumference, cm	91.2 (13.4)
Waist-hip ratio	0.86 (80.3–91.3)
Cardiovascular agents	
Antihypertensives	48.3
Statins	28.4
Ezetimibe	0.9
Oral glucose-lowering agents	8.9
Insulin	1.7
RA characteristics	
Disease duration, yrs	13.6 (9.3)
Rheumatoid factor-positive	75.8
Clinical Disease Activity Index	7.3 (2.0–13.8)
Disease Activity Score in 28 joints	3.9 (1.5)
Swollen joints	1 (0–4)
Tender joints	0 (0–2)
Patient disease activity	3.3 (2.8)
Physician disease activity	1.2 (0.0–3.0)
Erythrocyte sedimentation rate, mm/h	12 (5–27)
C-reactive protein, mg/l	5.3 (2.2–12.5)
Interleukin 6, pg/ml	3.5 (2.2–5.9)
Leukocytes, n/nl	5.9 (4.8–7.6)
No. deformed joints	73.6
Extraarticular manifestation	7.6
Synthetic disease-modifying agents	
Methotrexate	84.3
Chloroquine	66.1
Leflunomide	19.7
Sulfasalazine	19.1
Azathioprine	14.8
Tetracycline	11.9
Cyclophosphamide	3.4
Penicillamine	3
Number	2.4 (0.9)
Prednisone use	2.5
Tumor necrosis factor-α blockade	3.8
Glomerular filtration rate, ml/min/1.73 m^2	97 (81–114)
Framingham score	2 (1–6)
Metabolic risk factors	
Conventional	
Hypertension	59.3
Systolic blood pressure, mmHg	134 (22)
Diastolic blood pressure, mmHg	82 (13)
Total cholesterol, mmol/l	4.8 (1.0)
HDL cholesterol, mmol/l	1.53 (1.30–1.89)
LDL cholesterol, mmol/l	2.7 (0.8)
Triglycerides, mmol/l	1.0 (0.8–1.4)
Cholesterol-HDL cholesterol ratio	3.2 (1.0)
Cholesterol-HDL cholesterol ratio > 4	18.2
Non-HDL cholesterol, mmol/l	3.2 (1.0)
Diabetes	12.3
Glucose, mmol/l	4.7 (4.4–5.2)
Adipokines	
Leptin, pg/ml	10,195 (5463– 18,531)
Total adiponectin, μg/ml	7.36 (4.82–12.19)
High molecular adiponectin, μg/ml	3.26 (1.72–5.77)
Leptin-adiponectin ratio	1376 (548–2684)
Resistin, ng/ml	33.9 (22.7–53.1)
Retinol binding protein 4, μg/ml	19,846 (37,275)
Chemerin, ng/ml	114 (35)
Endothelial activation molecules	
Early endothelial activation	
E-selectin, ng/ml	39.1 (18.6)
VCAM-1, ng/ml	833 (667–1041)
ICAM-1, ng/ml	274 (211–352)
MCP-1, pg/ml	424 (265–679)
Angiopoietin 2, pg/ml	2560 (2044–3341)
Carotid atherosclerosis	
Intima-media thickness, mm	0.708 (0.110)
Plaque	40.3

RA: rheumatioid arthritis; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VCAM-1: vascular adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; MCP-1: monocyte chemoattractant protein 1.

strongly associated with angiopoietin 2 levels. Further, chemerin concentrations were related to cIMT and plaque in univariate and multivariate analysis, respectively. In a separate analysis, the chemerin concentrations-plaque relationship became significant only once leptin levels were added to the model (data not shown).

The presence of major cardiovascular risk factors affected the chemerin-selectin concentration, chemerin-endothelial activation score, and chemerin-plaque relationship (interaction p = 0.05, 0.03, and 0.02, respectively); generalized obesity affected the chemerin-plaque association (interaction p = 0.01); and abdominal obesity affected the chemerin-ICAM-1 and chemerin-cIMT relationship (interaction p = 0.02 and 0.04, respectively).

Table 4 gives results obtained in the related stratified analysis. Chemerin concentrations were related to the endothelial activation score in patients with but not in those without major cardiovascular risk factors, with cIMT in patients that were overweight or obese, and those who sustained abdominal obesity but not in those without these risk factors, and with plaque in patients without but not in those with generalized obesity. The β (SE) for the chemerin-cIMT relations in patients who were overweight or obese and those who experienced abdominal obesity were

Table 2. Significant associations of baseline characteristics with chemerin concentrations and of the latter with metabolic risk factors. The baseline variable-chemerin associations were assessed in demographic characteristics adjusted models. The chemerin-metabolic risk factor relations were evaluated in demographic characteristics as well as waist circumference, glomerular filtration rate, use of leflunomide and tumor necrosis factor-α blockade, cardiovascular drug use, and C-reactive protein concentrations adjusted models.

Characteristics	β (SE)	p
Demographic characteristics		
Age at study time	0.405 (0.209)	0.05
Female sex	12.5 (6.2)	0.04
Black	13.5 (4.4)	0.002
Anthropometry		
Body mass index	1.55 (0.39)	< 0.0001
Waist circumference	0.75 (0.17)	< 0.0001
Cardiovascular agents		
Antihypertensives	12.3 (4.6)	0.009
RA characteristics		
Disease Activity Score in 28 joints	3.97 (1.51)	0.009
Physician disease activity*	22.0 (7.8)	0.005
Erythrocyte sedimentation rate*	19.0 (5.2)	0.0003
C-reactive protein*	22.3 (4.0)	< 0.0001
Leukocytes*	43.2 (15.3)	0.005
Glomerular filtration rate*	–57.4 (17.9)	0.002
Metabolic risk factors		
Adipokines		
Leptin*	0.002 (0.001)	0.008

*Logarithmically transformed variables. –β: regression coefficient; SE: standard error; RA: rheumatoid arthritis.

larger than in those without these characteristics ($p < 0.0001$ and 0.04, respectively). Chemerin concentrations were consistently associated with those of angiopoietin 2 among subgroups.

In the models shown in Table 4, we adjusted for the Framingham score, which includes age. Nevertheless, the confounding effect of age may have been underestimated. When we replaced the Framingham score by age, sex, hypertension, dyslipidemia, smoking, and diabetes, the chemerin-cIMT relationship remained significant in patients with generalized obesity, overweight, or generalized obesity and abdominal obesity [β (SE) = 0.001 (0.0004), p = 0.01, 0.001 (0.0003), p = 0.02 and 0.001 (0.0003), p = 0.002, respectively]. This approach was not to be used for chemerin-plaque relations because it would have meant using overfitted logistic regression models, which can give false-positive or false-negative results.

DISCUSSION

In this RA study, chemerin concentrations were consistently and independently associated with those of angiopoietin 2, which contributes relevantly to angiogenesis[34], and hence is an endothelial activation of advanced rather than early atherosclerosis[11]. Angiopoietin 2 concentrations are related to prevalent and incident CVD in RA[35,36].

Chemerin concentrations were also independently associated to carotid artery plaque in all patients. Interestingly, this relationship was leptin-driven, and hence suggests a combined effect of leptin and chemerin in atherosclerosis among patients with RA. However, our most striking finding was that the presence of major conventional cardiovascular risk factors, excess abdominal and generalized adiposity, and RA severity markers consistently modified the independent chemerin-cardiovascular risk relations. This translated into associations of chemerin concentrations with cIMT in patients who were overweight or had generalized obesity and those who experienced abdominal obesity, and with carotid artery plaque in patients who were normal or overweight.

Table 3. Relationships of chemerin concentrations with endothelial activation and carotid atherosclerosis in 236 patients. Framingham score, race, waist circumference, glomerular filtration rate, leflunomide and tumor necrosis factor-α blockade use, and C-reactive protein and leptin concentrations were adjusted for in multivariate models. Significant association is shown in bold.

	Univariate		Multivariate	
Characteristics	β (SE)	p	β (SE)	p
Endothelial activation				
Early				
E-selectin	0.059 (0.036)	0.1	0.012 (0.042)	0.8
VCAM-1*	0.0004 (0.0003)	0.1	0.0004 (0.0003)	0.2
ICAM-1*	–0.0004 (0.0003)	0.2	–0.0001 (0.0004)	0.7
MCP-1*	0.001 (0.001)	0.09	0.001 (0.001)	0.1
Early endothelial activation score	0.007 (0.004)	0.1	0.006 (0.005)	0.2
Angiopoietin 2*	**0.002 (0.0004)**	**< 0.0001**	**0.002 (0.0004)**	**0.0002**
Atherosclerosis				
Intima-media thickness	**0.0005 (0.0002)**	**0.02**	0.0004 (0.0002)	0.1
	OR (95% CI)	p	OR (95% CI)	p
Plaque	1.005 (0.999–1.011)	0.08	**1.006 (1.000–1.013)**	**0.05**

*Logarithmically transformed. β: regression coefficient; SE: standard error; VCAM-1: vascular adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; MCP-1: monocyte chemoattractant protein 1.

Table 4. Independent relationships of chemerin concentrations (range = 2 to 282 ng/ml) with endothelial activation and atherosclerosis in subgroups. Framingham score, race, waist circumference, glomerular filtration rate, leflunomide and tumor necrosis-α blockade use, and C-reactive protein and leptin concentrations were adjusted for in each model. Significant associations are shown in bold.

Subgroups ≥ 1 major CVRF	Endothelial Activation Score β (SE)	p	Angiopoietin 2 Concentrations β (SE)	p	Intima-Media Thickness β (SE)	p	Plaque OR (95% CI)	p
Yes, n = 162	**0.011 (0.006)**	**0.04**	**0.001 (0.0004)**	**0.006**	0.0004 (0.0003)	0.08	1.008 (0.999–1.098)	0.06
No, n = 66	0.0007 (0.0007)	0.3	**0.002 (0.001)**	**0.001**	0.0002 (0.0005)	0.4	1.005 (0.988–1.022)	0.5
Missing, n = 8								
Obesity								
Yes, n = 70	0.001 (0.009)	0.9	**0.002 (0.001)**	**0.007**	**0.001 (0.0003)**	**0.008**	1.003 (0.990–1.017)	0.6
No, n = 161	0.007 (0.007)	0.3	**0.002 (0.001)**	**0.001**	0.0001 (0.0003)	0.7	**1.008 (1.000–1.016)**	**0.03**
Missing, n = 5								
Overweight or obese								
Yes, n = 138	0.004 (0.007)	0.5	**0.002 (0.001)**	**0.005**	**0.001 (0.0003)**	**0.005**	1.006 (0.997–1.015)	0.2
No, n = 93	0.009 (0.010)	0.4	**0.001 (0.001)**	**0.02**	–0.001 (0.0004)	0.2	1.005 (0.995–1.016)	0.3
Missing, n = 5								
MetS waist								
Yes, n = 105	–0.004 (0.008)	0.6	**0.001 (0.001)**	**0.01**	**0.001 (0.0001)**	**0.001**	1.005 (0.995–1.016)	0.3
No, n = 128	0.013 (0.008)	0.09	**0.002 (0.001)**	**0.007**	–0.0002 (0.0004)	0.6	1.009 (0.999–1.018)	0.06
Missing, n = 3								

β: regression coefficient; SE: standard error; CVRF: cardiovascular risk factors (hypertension, dyslipidemia, smoking, and diabetes); MetS: metabolic syndrome.

Maradit Kremers, *et al*[37] and Escalante, *et al*[38] reported a paradoxical protective effect of BMI against ischemic heart disease and overall mortality, respectively, in patients with RA. A modulating effect of systemic inflammation on the influence of body mass on cardiovascular risk and mortality was implicated. Abdominal adiposity also relates paradoxically to reduced endothelial activation in RA[39].

Importantly in the present context, cIMT and plaque are different phenotypes of atherosclerosis, and biologically and genetically distinct[40,41,42,43,44,45]. Intima-media thickening reflects mostly an enlarged tunica media due to an adapted response to aging and particularly blood pressure[41], and is related to stroke risk factors and prevalence[44,45]. By contrast, carotid plaque develops because of intimal pathology[40] and represents advanced atherosclerosis that is more closely linked to coronary artery disease risk factors and prevalence[41,44]. We have documented that BMI or generalized adiposity associates independently with blood pressure and cIMT, whereas anthropometric measures of abdominal adiposity are related to lipids and carotid artery plaque in women with intensively managed RA[46]. Thus, BMI and the waist-hip ratio are associated with different metabolic risk factor profiles and reflect different aspects of atherosclerosis in RA. Also, the INTERHEART study investigators[47] found that the waist-hip ratio was more strongly associated with myocardial infarction than was BMI, whereas in a large study performed in Finland and reported by Hu, *et al*[48], BMI but not waist-hip ratio enhanced the risk of stroke in women. Notably, coronary artery but not cerebrovascular event rates were included as outcome characteristics in the Maradit Kremers, *et al* study[37].

In our previous investigation on adiposity and atherosclerosis in RA, the relationships of BMI with cIMT and waist-hip ratio with plaque were explained by metabolic risk factors[46]. Conversely, in our current study, the independent relationship between chemerin and atherosclerosis in both obese and nonobese participants was not explained by metabolic risk factors, as well as several other potential mediating and confounding characteristics. Taken together, our findings indicate that chemerin represents CVD risk in both obese and nonobese patients with RA. Additionally, our results suggest that chemerin may contribute to the link between inflammation and increased risk of coronary artery disease among nonobese patients with RA[37]. Indeed, systemic inflammation was strongly associated with chemerin concentrations in our analysis, and suppression of inflammation with adalimumab is linked to reduced chemerin concentrations in RA[15]. Nevertheless, it remains uncertain whether our findings show a unique link between chemerin and CVD and that chemerin thereby contributes to the excess atherosclerosis burden in RA because the present investigation did not include patients without RA. Future studies should determine the association of chemerin concentrations with prevalent and incident cerebrovascular and coronary artery disease in obese and nonobese patients with RA, as well as in patients without RA.

In the recent study by Gonzalvo-Feo, *et al*[49], retinoic acid-activated endothelial cells were shown to promote dendritic cell transmigration across endothelial cell monolayers through the endogenous production of chemerin, upregulation of CCRL2, and activation of dendritic cell β1 integrin affinity. Inflammatory cytokines are markedly

overproduced in RA and can induce the production of retinoic acid[49]. The potential role of chemerin derived from endothelial cell activation in atherogenesis merits further study.

Among patients with major conventional risk factors, chemerin was associated not only with angiopoietin 2 concentrations, but also with surrogate markers of early endothelial activation in our study. These patients also had an increased OR for plaque, but this did not reach significance ($p = 0.06$). The presence of major conventional risk factors also modifies the potential effects of resistin on surrogate markers of early endothelial activation in RA[19].

RA severity additionally affected the chemerin-conventional metabolic risk relations, but in contrast to findings on resistin in atherogenesis, disease severity modified neither the chemerin-endothelial activation associations nor the chemerin-atherosclerosis relation. Leptin is an adiposity marker in RA[18], and in our study, both leptin and chemerin concentrations were higher in obese compared to nonobese patients (data not shown). Reminiscent of the paradoxical effect of adiposity on cardiovascular risk[37], we found that chemerin concentrations are independently related to those of leptin in nonobese rather than obese patients. Whereas chemerin concentrations reportedly predict metabolic risk development in patients without RA[8], we did not find an association of chemerin concentrations with prevalent conventional metabolic risk factors.

A recent South Korean study performed in patients with RA revealed that disease activity rather than obesity was associated with chemerin concentrations in RA[50]. We found that disease activity and adiposity were related to chemerin concentrations independent of one another. We also showed that chemerin concentrations are higher in African black patients compared to white patients with RA. In this regard, we previously reported that both the production and biological effects of adipokines can differ by population or ethnic origin in RA[20,22].

Besides angiopoietin 2 concentrations, we assessed the production of 4 endothelial activation molecules that mediate the initial stages of atherosclerosis, and were shown to be strongly upregulated and associated with prevalent and incident atherosclerosis in RA[51,52]. CIMT and carotid artery plaque that were our main outcome measures independently predicted incident cardiovascular events in patients with RA and patients without RA[53,54].

Our study has limitations. Conceptually, endothelial activation marker concentrations can represent disease activity. The chemerin-endothelial activation relationship identified in the present investigation were independent of disease activity variables. Our cross-sectional study design precludes drawing inferences on the direction of causality. Circulating CRP concentrations are determined not only by disease activity, but also by insulin resistance in RA[55], and those of chemerin do not necessarily represent its tissue levels[8]. We did not include markers of insulin resistance such as the homeostasis model assessment of insulin resistance, which could have been informative in the present context. The standard z-score of endothelial activation as used in our study was previously reported by us[18,19,22,39], but nevertheless awaits external validation.

Contrary to other investigated adipokines, apart from retinol binding protein 4, chemerin relates not only to surrogate markers of endothelial activation, but also atherosclerosis in RA. Chemerin represents CVD risk and adiposity influences the chemerin-atherosclerotic phenotype relations in RA. Additional longitudinal studies are needed to fully determine the role of chemerin concentrations in cardiovascular risk stratification in RA.

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REFERENCES

1. Dessein PH, Norton GR, Woodiwiss AJ, Joffe BI, Wolfe F. Influence of nonclassical cardiovascular risk factors on the accuracy of predicting subclinical atherosclerosis in rheumatoid arthritis. J Rheumatol 2007;34:943-51.
2. Solomon DH, Kremer J, Curtis JR, Hochberg MC, Reed G, Tsao P, et al. Explaining the cardiovascular risk associated with rheumatoid arthritis: traditional risk factors versus markers of rheumatoid arthritis severity. Ann Rheum Dis 2010;69:1920-5.
3. Nurmohamed M, Kitas G. Cardiovascular risk in rheumatoid arthritis and diabetes: how does it compare and when does it start? Ann Rheum Dis 2010;70:881-3.
4. Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P, Kvien TK, et al. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. Ann Rheum Dis 2010;69:325-31.
5. Crowson CS, Gabriel SE. Towards improving cardiovascular risk management in patients with rheumatoid arthritis: the need for accurate risk assessment. Ann Rheum Dis 2011;70:719-21.
6. Dessein PH, Semb AG. Could cardiovascular disease risk stratification and management in rheumatoid arthritis be enhanced? Ann Rheum Dis 2013;72:1743-6.
7. Yamawaki H. Vascular effects of novel adipocytokines: focus on vascular contractility and inflammatory responses. Biol Pharm Bull 2011;34:307-10.
8. Rourke JL, Dranse HJ, Sinal CJ. Towards an integrative approach to understanding the role of chemerin in human health and disease. Obesity Rev 2013;14:245-62.
9. Landgraf K, Friebe D, Ullrich T, Kratzsch J, Dittrich K, Herberth F, et al. Chemerin as a mediator between obesity and vascular inflammation in children. J Clin Endocrinol Metab 2012;97: E556-64.
10. Bozaoglu K, Curran JE, Stocker CJ, Zaibi MS, Segal D, Konstantopoulos N, et al. Chemerin, a novel adipokine in the regulation of angiogenesis. J Clin Endocrinol Metab 2010; 95:2476-85.
11. Khurana R, Simons M, Martin JF, Zachary IC. Role of angiogenesis in cardiovascular disease: a critical appraisal. Circulation 2005;112:1813-24.
12. Gomez R, Conde J, Scotece M, Gomez-Reino JJ, Lago F, Gualillo O. What's new in our understanding of the role of adipokines in rheumatic diseases? Nat Rev Rheumatol 2011;7:528-36.

13. Kaneko K, Miyabe Y, Takayasu A, Fukuda S, Miyabe C, Ebisawa M, et al. Chemerin activates fibroblast-like synoviocytes in patients with rheumatoid arthritis. Arthritis Res Ther 2011;13:R158.
14. Eisinger K, Bauer S, Schaffler A, Walter R, Neumann E, Buechler C, et al. Chemerin induces CCL2 and TLR4 in synovial fibroblasts of patients with rheumatoid arthritis and osteoarthritis. Exp Mol Pathol 2012;92:90-6.
15. Herenius MM, Oliveira AS, Wijbrandts CA, Gerlag DM, Tak PP, Lebre MC. Anti-TNF therapy reduces serum levels of chemerin in rheumatoid arthritis: a new mechanism by which anti-TNF might reduce inflammation. PLoS ONE 2013;8:e57802.
16. Gonzalez-Gay MA, Llorca J, Garcia-Unzueta MT, Gonzalez-Juanatey C, De Matias JM, Martin J, et al. High-grade inflammation, circulating adiponectin concentrations and cardiovascular risk factors in severe rheumatoid arthritis. Clin Exp Rheumatol 2008;26:596-603.
17. Gonzalez-Gay MA, Garcia-Unzueta MT, Berja A, Gonzalez-Juanatey C, Miranda-Filloy JA, Vazquez-Rodriguez TR, et al. Anti-TNF-alpha therapy does not modulate leptin in patients with severe rheumatoid arthritis. Clin Exp Rheumatol 2009; 27:222-8.
18. Dessein PH, Norton GR, Badenhorst M, Woodiwiss AJ, Solomon A. Rheumatoid arthritis impacts on the independent relationships between circulating adiponectin concentrations and cardiovascular metabolic risk. Mediators Inflamm 2013;2013:461849.
19. Dessein PH, Norton GR, Woodiwiss AJ, Solomon A. Independent relationship between circulating resistin concentrations and endothelial activation in rheumatoid arthritis. Ann Rheum Dis 2013;72:1586-8.
20. Dessein PH, Solomon A. Towards the elucidation of the true impact of adipocytokines on cardiovascular risk in rheumatoid arthritis. Arthritis Res Ther 2013;15:127.
21. Dessein PH, Norton GR, Woodiwiss AJ, Tsang L, Solomon A. Age impacts on the independent relationships of leptin with cardiometabolic risk and surrogate markers of enhanced early atherogenesis in black and white patients with rheumatoid arthritis: a cross-sectional study. Rheumatol Int 2014;34:329-39.
22. Dessein PH, Woodiwiss AJ, Norton GR, Tsang L, Solomon A. Independent associations of total and high molecular weight adiponectin with cardiometabolic risk and surrogate markers of enhanced early atherogenesis in black and white patients with rheumatoid arthritis: a cross-sectional study. Arthritis Res Ther 2013;15:R128.
23. Popa C, Netea MG, de Graaf J, van den Hoogen FH, Radstake TR, Toenhake-Dijkstra H, et al. Circulating leptin and adiponectin concentrations during tumor necrosis factor blockade in patients with active rheumatoid arthritis. J Rheumatol 2009;36:724-30.
24. Gonzalez-Gay MA, Garcia-Unzueta MT, Gonzalez-Juanatey C, Miranda-Filloy JA, Vazquez-Rodriguez TR, De Matias JM, et al. Anti-TNF-alpha therapy modulates resistin in patients with rheumatoid arthritis. Clin Exp Rheumatol 2008;26:311-6.
25. Ferraz-Amaro I, González-Juanatey C, López-Mejias R, Riancho-Zarrabeitia L, González-Gay MA. Metabolic syndrome in rheumatoid arthritis. Mediators of Inflamm 2013;2013:710928.
26. Dessein PH, Tsang L, Norton GR, Woodiwiss AJ, Solomon A. Retinol binding protein 4 concentrations relate to enhanced atherosclerosis in obese patients with rheumatoid arthritis. PLoS One 2014;9:e92739.
27. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
28. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010;69:1580-8.
29. Gepner AD, Korcarz CE, Aeschlimann SE, LeCaire TJ, Palta M, Tzou WS, et al. Validation of a carotid intima-media thickness border detection program for use in an office setting. J Am Soc Echocardiogr 2006;19:223-8.
30. Dessein PH, Joffe BI, Veller MG, Stevens BA, Tobias M, Reddi K, et al. Traditional and nontraditional cardiovascular risk factors are associated with atherosclerosis in rheumatoid arthritis. J Rheumatol 2005;32:435-42.
31. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc Dis 2007;23:75-80.
32. Dessein PH, Tobias M, Veller MG. Metabolic syndrome and subclinical atherosclerosis in rheumatoid arthritis. J Rheumatol 2006;33:2425-32.
33. Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation 2002;106:3143-421.
34. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 1997;277:55-60.
35. Lopez-Mejias R, Corrales A, Genre F, Hernandez JL, Ochoa R, Blanco R, et al. Angiopoietin-2 serum levels correlate with severity, early onset and cardiovascular disease in patients with rheumatoid arthritis. Clin Exp Rheumatol 2013;31:761-6.
36. Westra J, de Groot L, Plaxton SL, Brouwer E, Posthumus MD, Kallenberg CG, et al. Angiopoietin-2 is highly correlated with inflammation and disease activity in recent-onset rheumatoid arthritis and could be predictive for cardiovascular disease. Rheumatology 2011;50:665-73.
37. Maradit Kremers H, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE. Prognostic importance of low body mass index in relation to cardiovascular mortality in rheumatoid arthritis. Arthritis Rheum 2004;50:3450-7.
38. Escalante A, Haas RW, del Rincon I. Paradoxical effect of body mass index on survival in rheumatoid arthritis. Arch Intern Med 2005;165:1624-9.
39. Dessein PH, Solomon A, Woodiwiss AJ, Norton GR, Tsang L, Gonzalez-Gay MA, et al. Marked independent relationship between circulating interleukin-6 concentrations and endothelial activation in rheumatoid arthritis. Mediator Inflamm 2013;2013:510243.
40. Simon A, Megnien JL, Chironi G. The value of carotid intima-media thickness for predicting cardiovascular risk. Arterioscler Thromb Biol 2010;30:182-5.
41. Riccio SA, House AA, Spence JD, Fenster A, Parraga G. Carotid ultrasound phenotypes in vulnerable populations. Cardiovasc Ultrasound 2006;4:44.
42. Johnsen SH, Mathiesen EB, Joakimsen O, Stensland E, Wilsgaard T, Løchen ML, et al. Carotid atherosclerosis is a stronger predictor of myocardial infarction in women than in men: a 6-year follow-up study of 6226 persons: The Tromsø. Stroke 2007;38:2873-80.
43. Ebrahim S, Papacosta O, Whincup P, Wannamethee G, Walker M, Nicolaides AN, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. Stroke 1999;30:841-50.
44. Spence JD, Hegele RA. Noninvasive phenotypes of atherosclerosis. Arterioscler Thromb Vasc Biol 2004;24:e188.
45. Johnsen SH, Mathiesen EB. Carotid plaque compared with intima-media thickness as a predictor of coronary and

cerebrovascular disease. Curr Cardiol Rep 2009;11:21-7.
46. Solomon A, Norton GR, Woodiwiss AJ, Dessein PH. Obesity and carotid atherosclerosis in African black and Caucasian women with established rheumatoid arthritis: a cross-sectional study. Arthritis Res Ther 2012;14:R67.
47. Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi G, Commerford P, et al, on behalf of the INTERHEART Study Investigators. Obesity and the risk of myocardial infarction in 27 000 participants from 52 countries: a case-control study. Lancet 2005;366:1640-9.
48. Hu G, Tuomilehto J, Silventoinen K, Sartri C, Mannisto S, Jousilahti P. Body mass index, waist circumference, and waist-hip ratio on the risk of total and type-specific stroke. Arch Intern Med 2007;167:1420-7.
49. Gonzalvo-Feo S, Del Prete A, Pruenster M, Salvi V, Wang L, Sironi M, et al. Endothelial cell-derived chemerin promotes dendritic cell transmigration. J Immol 2014;192:2366-73.
50. Ha YJ, Kang EJ, Song JS, Park YB, Lee SK, Choi ST. Plasma chemerin levels in rheumatoid arthritis are correlated with disease activity rather than obesity. Joint Bone Spine 2013;81:189-90.
51. Dessein PH, Joffe BI, Singh S. Biomarkers of endothelial dysfunction, cardiovascular risk factors and atherosclerosis in rheumatoid arthritis. Arthritis Res Ther 2005;7:R634-43.
52. Sodergren A, Karp K, Boman K, Eriksson C, Lundstrom E, Smedby T, et al. Atherosclerosis in early rheumatoid arthritis: very early endothelial activation and rapid progression of intima media thickness. Arthritis Res Ther 2010;12:R158.
53. Gonzalez-Juanatey C, Llorca J, Martin J, Gonzalez-Gay MA. Carotid intima-media thickness predicts the development of cardiovascular events in patients with rheumatoid arthritis. Semin Arthritis Rheum 2009;38:366-71.
54. Evans MR, Escalante A, Battafarano DF, Freeman GL, O'Leary DH, del Rincon I. Carotid atherosclerosis predicts incident acute coronary syndrome in rheumatoid arthritis. Arthritis Rheum 2011;63:1211-20.
55. Dessein PH, Norton GR, Woodiwiss AJ, Joffe BI, Solomon A. Independent role of conventional cardiovascular risk factors as predictors of C-reactive protein concentrations in rheumatoid arthritis. J Rheumatol 2007;34:681-8.