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BRIEF REPORT

The cross-sectional association between chemerin and bone health in peri/pre and postmenopausal women: results from the EPIC-Potsdam study

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Abstract

Objective: Recent in vitro data suggested that the novel adipokine chemerin may influence bone health. However, only limited evidence of the relationship between chemerin and bone health in humans is available. Therefore, this study aimed to investigate the association between chemerin and broadband ultrasound attenuation (BUA) in peri/premenopausal and postmenopausal women.

Methods: Data from the German population-based European Prospective Investigation into Cancer and Nutrition-Potsdam cohort comprising 404 peri/premenopausal and 279 postmenopausal women were analyzed. Multivariable-adjusted analysis of covariance including age, body mass index, waist circumference, smoking status, education, physical activity, alcohol consumption, and hormone use was used to investigate potential relationships between the adipokine and BUA levels in peri/premenopausal and postmenopausal women, respectively.

Results: The concentrations of chemerin were lower in peri/premenopausal women (median 118.0 ng/mL, interquartile range [IQR] 99.2-135.0), compared with postmenopausal women (median 140.0 ng/mL, IQR 121.0-167.0). In peri/premenopausal women chemerin was inversely associated with BUA levels; after multivariable adjustment, a 10% increase in the chemerin concentration was significantly associated with 0.83 dB/MHz lower BUA levels ($P = 0.0006$). In postmenopausal women chemerin was not related to BUA levels ($P = 0.8$).

Conclusion: The present study provides evidence for an inverse association between chemerin and BUA in peri/premenopausal women. Therefore, the study suggests that high chemerin concentrations may minimize peak bone mass and thereby may promote age-related bone loss. Further studies are needed to investigate the role of chemerin in bone homeostasis in peri/premenopausal and postmenopausal women.

Key Words: Adipokines – Bone mineral density – Broadband ultrasound attenuation – BUA – Menopause.

Bone health is characterized by a continuous remodeling process, that is, removal of mineralized bones by osteoclasts, followed by the formation of new bone through the osteoblasts.[1] During menopause transition in women, this balance shifts, favoring greater bone resorption and less bone formation, thus inducing accelerated bone loss in menopause, and ultimately results in higher risk of osteoporosis and fractures.[1] It is known that intrinsic and extrinsic factors accelerate the decline in bone mass. Intrinsic factors include genetics or peak bone mass accrual in youth.[1] Extrinsic factors include, for instance, physical activity, nutrition, or intake of medicinal drugs.[1] Interestingly, recent evidence

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suggested that adipokines, bioactive molecules released by adipose tissue, might also offer a possible connection between adipose tissue and bone health. First suggested by leptin, showing that leptin promotes osteoblast differentiation in human marrow stromal cells.[2]

Nowadays, it is being suggested that the novel adipokine chemerin may also influence bone health. Chemerin, also known as tazarotene-induced gene 2 (TIG2) or retinoic acid receptor responder protein 2 (RARRES2), is a 14-kDa protein secreted in an inactive form and is activated through cleavage of the C-terminus by serine proteases. Recent in vitro data provide evidence that chemerin is negatively associated with bone metabolism, showing that knockdown of chemerin gene in bone marrow stromal cells (BMSCs) increased osteoblast marker gene expression and mineralization.[3] Moreover, neutralization of chemerin resulted in a near-complete loss of osteoclastogenesis by reduced osteoclast marker gene expression, for example, tartrate-resistant acid phosphatase or matrix metallopeptidase 9, and resorption.[4]

Yet, the relationship between concentrations of chemerin and bone mineral density (BMD) has not been extensively investigated in humans.[5-7] Therefore, the present study aimed to investigate the association between chemerin and broadband ultrasound attenuation (BUA), as one parameter reflecting bone health, in a cross-sectional study using data from a population-based sample of German women. Based on the physiological accelerated bone loss occurring after menopause, all the analyses were conducted separately in peri/premenopausal and postmenopausal women to reveal potential different associations.

METHODS

Study population

The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study is part of the multicenter prospective cohort study conducted in 10 European countries.[8] Between 1994 and 1998, 27,548 participants (10,904 men and 16,644 women) were randomly selected from the general population of Potsdam and surrounding communities.[8] From 1996 to 1998, quantitative ultrasound measurements (QUS) were part of the baseline examination in women (n = 9,711). Chemerin concentrations were measured in a random subsample of 1,002 out of the 9,711 women with already measured BUA levels. For the present study, participants were excluded due to: age at baseline below 35 years (n = 11), uncertain menopausal status (n = 255), surgical menopausal status (n = 39), and postmenopausal women taking oral contraceptives (n = 2) or with missing covariate data (n = 12). The final study population consisted of 683 women—in detail 404 peri/premenopausal and 279 postmenopausal women.

Quantitative ultrasound measurement

Quantitative ultrasound measurements were performed by trained personnel on the right os calcis using Achilles Plus Ultrasound Densitometer (Lunar Corporation, Madison, WI) according to manufacturer's instructions.[9] BUA was measured in decibel/ megahertz (dB/MHz), defined as the slope of the signal attenuation versus the frequency curve in the usually measured range of 0.1 to 1 MHz.[10] A substudy (n = 11 women) observed a within-person variation coefficient of 1.47% of BUA, when BUA was measured 10 times within 3 weeks.[11]

Exposure and covariate measurements

A total of 30 mL of venous blood was collected at baseline (1994-1998) from participants at the Potsdam study center. Blood was fractionated into serum, plasma, buffy coat, and erythrocytes, and stored in the vapor phase of liquid nitrogen (−196°C) or in freezers (−80°C) for conservation until time of analysis.[8] In 2015, in the random subcohort of the EPIC-Potsdam study, the concentration of chemerin was measured in plasma samples using a sandwich ELISA by Biovendor (Brno, Czech Republic) at the Institute for Clinical Chemistry and Pathobiochemistry, Otto-von-Guericke University Magdeburg (Magdeburg, Germany). Chemerin was measured with intra-assay coefficients of variation (CVs) between 5.1% and 7.0%, interassay CVs between 6.9% and 8.3%, and a lower limit of detection of 0.1 ng/mL.

At baseline, trained personnel took the anthropometric measurements (weight, height), with participants wearing light underwear and no shoes, with a precision of 0.1 kg and 0.1 cm, respectively. Body weight was measured with electronic digital scales (Soehnle, type 67720/23, Murrhardt, Germany) and height by using a flexible anthropometer. Body mass index (BMI) was calculated as body weight (kg) divided by squared height (m^2). Waist circumference was measured midway between the lower ribs and the iliac crest. Information on educational level, smoking habits, and medical and reproductive history was obtained by computer-assisted face-to-face interviews at baseline.[12] In detail, self-reported medication over the 4 weeks before study enrolment was used to identify users of oral contraceptive and hormone therapy (HT). Menopausal status was assessed according to self-reported information on menstrual status and history, for example, current menstruation, menstruation in the past 12 months, surgery (hysterectomy, ovariectomy), HT, and so on.[9,13] Dietary habits including alcohol consumption were also part of the baseline examination, assessed by a validated food frequency questionnaire.[8] Moreover, calibrated baseline EPIC-Germany physical activity data were used based on a comprehensive physical activity questionnaire and objectively measured acceleration counts.[13,14]

Statistical analysis

All analyses were performed separately for peri/premenopausal women (n = 404) and postmenopausal women (n = 279). Normally distributed variables were reported as mean and standard derivation. Concentration of chemerin and alcohol consumption was skewed, thus reported as median and interquartile range (IQR), and log-transformed for the analyses. Categorical variables were reported as percentages (smoking status, educational level, use of oral contraceptives or HT). For comparison of the characteristics between peri/

TABLE 1. *Characteristics of the study population according to menopausal status (EPIC-Potsdam study, women, N = 683)*

	Peri/premenopausal women (n = 404)	Postmenopausal women (n = 279)
BUA, dB/MHz[a]	112.4 ± 10.2	106.44 ± 9.95
Chemerin, ng/mL[b]	118.0 (99.2-135.0)	140.0 (121.0-167.0)
Age, y[a]	41.3 ± 4.5	59.4 ± 3.6
BMI, kg/m^2[a]	24.4 ± 4.6	27.5 ± 4.9
Waist circumference, cm[a]	77.5 ± 11.6	85.7 ± 11.1
Physical activity, counts/min/d[a]	40.8 ± 4.6	31.2 ± 5.2
Smoking status, %		
Nonsmoker	44.6	65.6
Ex-smoker	29.5	22.6
Smoker <20 cigarettes/d	20.3	10.0
Smoker ≥20 cigarettes/d	5.7	1.8
Educational level, %		
Unskilled or skilled	31.7	46.6
Technical college	26.2	33.7
University degree	42.1	19.7
Oral contraceptive intake, %	35.6	—
Hormone therapy, %	—	25.1
Alcohol consumption, g/d[b]	6.3 (2.2-11.5)	4.4 (1.1-8.7)

Lifestyle characteristics have been assessed during baseline examination (1994-1998). Venous blood was collected at baseline (1994-1998), fractionated, and stored until time of analysis. Chemerin concentration was measured in 2015. From 1996 to 1998, quantitative ultrasound measurements were part of the baseline examination in women only.
BMI, body mass index; BUA, broadband ultrasound attenuation.
[a]Mean and standard deviation.
[b]Median and interquartile range.

premenopausal women and postmenopausal women, a chi-square test for categorical variables and a Student's *t* test or Mann-Whitney *U* test for continuous variables were used. Multivariable linear regression models were used to estimate the associations between chemerin concentrations and BUA, adjusted for age (continuous), BMI (continuous), waist circumference (continuous), smoking status (nonsmoker, ex-smoker, smoker <20 cigarettes/d, smoker ≥20 cigarettes/d), educational level (unskilled or skilled, technical college, university degree), physical activity (continuous), alcohol consumption (continuous), use of oral contraceptive (yes/no), and HT (yes/no), respectively, for peri/premenopausal and postmenopausal women. Multivariable-adjusted analysis of covariance (ANCOVA) was used to assess the relationship between chemerin and BUA according to menopausal status-specific quartiles of chemerin. Multiplicative interactions between chemerin and obesity (waist circumference ≤/>88 cm; BMI ≤/>30 kg/m^2), HT, and oral contraceptive intake were tested with cross-product terms in the fully adjusted model in peri/premenopausal and postmenopausal women, respectively.

Sensitivity analyses were carried out, including the additional adjustment of the inflammatory marker high-sensitivity C-reactive protein (n = 628) or estradiol (n = 294). A *P* value <0.05 was considered to be statistically significant. All statistical analyses were performed using SAS software, version 9.4 (SAS institute, Cary, NC).

RESULTS

The distribution of general characteristics of the 683 women is shown in Table 1 according to menopausal status. The concentrations of chemerin were lower in peri/premenopausal women (median 118.0 ng/mL, IQR 99.2-135.0), compared with postmenopausal women (median 140.0 ng/mL, IQR 121.0-167.0). Moreover, peri/premenopausal women had higher BUA and PA levels (Table 1).

We observed an inverse association between chemerin and BUA in peri/premenopausal women. In particular, a 10% increase in chemerin concentrations was significantly associated with 0.83 dB/MHz lower BUA levels ($P = 0.0006$) after adjustment for age, BMI, waist circumference, smoking status, educational attainment, physical activity, alcohol consumption, and use of oral contraceptives (114.1; 95% confidence interval [CI] 111.8-116.5 dB/MHz vs 109.7; 95% CI 107.5-111.9 dB/MHz for the highest versus the lowest quartile of chemerin; *P* for trend = 0.005; Table 2). We observed no interactions between chemerin and BMI (*P* for interaction = 0.96), waist circumference (*P* for interaction = 0.8), or intake

TABLE 2. *Quartiles of chemerin with adjusted BUA values according to menopausal status*

Peri/premenopausal women (n = 404)				
	n	Chemerin, ng/mL Median (IQR)	BUA, dB/MHz[a] Adjusted mean (95% CI)	*P* for linear trend[c]
Q1	101	102.7 (95.6, 110.3)	114.1 (111.8, 116.5)	0.005
Q2	98	126.4 (119.5, 129.9)	112.2 (109.9, 114.4)	
Q3	107	147.3 (142.7, 153.1)	110.9 (108.8, 112.9)	
Q4	98	177.5 (165.9, 192.6)	109.7 (107.5, 111.9)	
Postmenopausal women (n = 279)				
	n	Chemerin, ng/mL Median (IQR)	BUA, dB/MHz[b] Adjusted mean (95% CI)	*P* for linear trend[c]
Q1	69	124.1 (114.0, 129.9)	106.7 (103.2, 110.1)	0.8
Q2	70	150.8 (146.2, 156.6)	105.7 (102.2, 109.2)	
Q3	71	176.3 (167.0, 185.6)	105.9 (102.6, 109.2)	
Q4	69	214.6 (201.8, 235.5)	107.2 (103.6, 110.8)	

BUA, broadband ultrasound attenuation; IQR, interquartile range.
[a]Adjusted for age, waist circumference, BMI, smoking status, education, physical activity, alcohol consumption, oral contraceptive.
[b]Adjusted for age, waist circumference, BMI, smoking status, education, physical activity, alcohol consumption, hormone therapy.
[c]*P* values for linear trend of BUA were assessed over quartiles of chemerin applying orthogonal polynomial contrasts.

of oral contraceptives (P for interaction = 0.4) in peri/premenopausal women.

In postmenopausal women (n = 279) we observed no association between chemerin and BUA (Table 2). The present study observed a significant interaction between chemerin and intake of HT (P for interaction = 0.03), but stratified analyses by intake of HT have not demonstrated a significant association between chemerin and BUA in women with HT intake (n = 70) (beta coefficient log-transformed chemerin = 6.4; $P = 0.3$) or without HT intake (n = 209) (beta coefficient log-transformed chemerin = −4.3; $P = 0.2$). Moreover, no further interactions were observed between chemerin and BMI (P for interaction = 0.8) or waist circumference (P for interaction = 0.9). Sensitivity analyses, including further adjustment for high-sensitivity C-reactive protein or estradiol, did not substantially alter the association between chemerin and BUA (data not shown).

DISCUSSION

To the best of our knowledge, this is the first study showing potential associations between chemerin and BUA in women of the general population, stratified by menopausal status. We observed a significant inverse association between chemerin concentrations and BUA in peri/premenopausal women, but no association between chemerin and BUA in postmenopausal women.

To date, the relationship between concentrations of chemerin and BMD has not been widely investigated in humans. Only few epidemiological studies have investigated the association between chemerin and BMD, suggesting an inverse association between chemerin and BMD. However, an appropriate detailed comparison to these studies is difficult because of differences in characteristics of the study populations, that is, Terzoudis et al[5] conducted the study in participants with inflammatory bowel diseases, Shi et al[6] restricted their study to obese women, and He et al[7] had a mixed study population of men and women (mean age 64 ± 10 years). Yet, there is no other study investigating the association between chemerin and bone health in women, strictly stratified by menopausal status.

Given the inverse association between chemerin and BUA in peri/premenopausal women in the present study, some experimental evidence provides plausible biological pathways for modulation of bone metabolism. It is well known that BMSCs can give rise to a variety of lineages, including osteoblasts, adipocytes, chondrocytes, and myocytes.[3] Interestingly, it is believed that extracellular signaling molecules, for example, chemerin, might cause one lineage to be favored over another.[3] Indeed, experimental evidence indicates that chemerin might promote toward adipocyte differentiation at the expense of osteoblastogenesis.[3] Accordingly, knockdown of chemerin or the cognate receptor chemokine-like receptor 1 in BMSCs showing increased osteoblast marker gene expression and mineralization suggests a chemerin/chemokine-like receptor 1 signaling pathway as negative regulator of osteoblastogenesis.[3] Moreover, chemerin also regulated osteoclast differentiation of hematopoietic stem cells, showing that chemerin neutralization resulted in a near-complete loss of osteoclastogenesis.[4] Therefore, we might propose that higher chemerin levels are associated with stronger inhibition of bone formation and stronger support of bone resorption. Given the inverse association between chemerin and BUA in peri/premenopausal women, the present study suggests that high chemerin concentrations may minimize the peak bone mass and thereby may promote age-related bone loss. In contrast, we observed no significant association between chemerin and BUA in postmenopausal women. The present study observed a significant interaction between chemerin and HT in postmenopausal women, but stratified analyses failed to provide associations between chemerin and BUA in women with or without HT intake. We suspect that larger sample sizes in the subgroups are required to supply reliable findings, and this is seen as a limitation. However, the interaction may provide first evidence that a possible relationship between chemerin and bone health might be affected by hormonal medication in postmenopausal women. All in all, we believe that our study could serve as a basis for future investigations regarding chemerin and bone health in women.

Some limitations of our study deserve to be mentioned. Firstly, BUA measurement, derived from QUS measurement, was used as a proxy of BMD measures. Dual-energy x-ray absorptiometry technique is the most frequently used technique for assessing bone mineral density. But BUA has been validated several times against this method, thus representing a valid, noninvasive, inexpensive, easy, and quick alternative assessment tool for bone health.[15] Secondly, the present findings are based on one single baseline measurement of chemerin, but we observed high reliability within individuals over time indicated by intraclass correlation coefficient of 0.72 (95% CI 0.65-0.78).[16] Third, the study included middle-aged German women, and therefore the results may not be generalized to other populations, such as other ethnic or age groups or men. Strengths of our study include the population-based sample with the availability of high-quality data as a result of the standardized procedures, ensuring a suitable comparison between peri/premenopausal and postmenopausal women in the same study population.

CONCLUSIONS

In conclusion, this study provides further evidence of an inverse association between chemerin and bone health in peri/premenopausal women. Therefore, the present study suggests that high chemerin concentrations may minimize the peak bone mass and thereby may promote age-related bone loss. Additional extensive studies are required to further investigate the role of chemerin in bone metabolism and whether chemerin is qualified as a biomarker of future osteoporosis.

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