Circulating Chemerin Level is Independently Correlated with Arterial Stiffness

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Aim: Adipokines have been implicated in the pathogenesis of obesity and obesity-related disorders, including atherosclerosis. Chemerin is a recently discovered adipokine which is closely correlated with various metabolic phenotypes in humans. We examined the association between circulating chemerin levels and arterial stiffness, as represented by the brachial ankle pulse wave velocity (baPWV).

Methods: Fifty-eight obese and 62 non-obese individuals participated in the study. We measured the serum chemerin and high sensitivity C-reactive protein (hsCRP) levels, and the homeostasis model assessment of insulin resistance (HOMA-IR), as well as other cardiovascular risk factors. Vascular health was assessed by the baPWV and carotid intima-media thickness (IMT).

Results: The serum chemerin level was significantly increased in obese individuals compared with lean controls (120.14 ± 19.43 ng/mL vs. 106.81 ± 23.39 ng/mL, p = 0.001). The circulating chemerin level had a significant positive correlation with the body mass index, waist circumference, HOMA-IR, and low-density lipoprotein-cholesterol, triglycerides, and hsCRP levels. The serum chemerin level was significantly associated with the baPWV (r = 0.280, p = 0.002), but not the carotid IMT (r = 0.065, p = 0.504). Multiple stepwise regression analysis showed that age (p < 0.001), waist circumference (p = 0.038), systolic blood pressure (p < 0.001), and serum fasting glucose (p = 0.003) and chemerin levels (p = 0.017) were definitive risk factors for arterial stiffness (r² = 0.457).

Conclusions: The circulating chemerin level was an independent risk factor for arterial stiffness even after adjusting for other cardiovascular risk factors.


Key words; Atherosclerosis, Chemerin, Pulse wave velocity

Introduction

Obesity is an important risk factor for atherosclerosis, but the underlying mechanism of this association is poorly understood. Adipose tissue serves as an active endocrine organ that secretes a number of hormone-like compounds, termed adipokines. Dysregulation of adipokine production caused by adipose tissue expansion provokes insulin resistance and systemic inflammation, contributing to the initiation of obesity-related disorders. Recently, some adipokines have been regarded as direct mediators between obesity and atherosclerosis by influencing the function of endothelial cells, arterial smooth muscle cells, and macrophages in the vessel wall[1]. The clarification of a novel adipokine which regulates the atherosclerotic process might provide new opportunities for developing more effective approaches for preventing cardiovascular disease.

Chemerin was originally identified as a chemotactant that promotes the recruitment of immature dendrite cells and macrophages to lymphoid organs and sites of tissue injury[2]. For the first time, Goralski et al.[3] discovered that chemerin was strongly expressed...
in white adipose tissue from mouse, rat, and human samples, and the secretion of chemerin from adipose tissue increased with adipocyte differentiation and obesity. Furthermore, Ernst et al.\(^6\) reported that exogenous administration of chemerin exacerbates glucose intolerance, lowers serum insulin levels, and decreases tissue glucose uptake in a mouse model of obesity and diabetes. Growing evidence derived from human data also supports a link among chemerin, obesity, and metabolic syndrome. A study involving a Mexican-American population reported that circulating chemerin levels are significantly higher in obese individuals compared with lean controls, and plasma chemerin levels are correlated positively with the body mass index (BMI), and fasting glucose, fasting insulin, and triglyceride levels, and negatively correlated with the high-density lipoprotein (HDL)-cholesterol level\(^5\). Sell et al.\(^6\) reported that in patients who had undergone bariatric surgery for weight reduction, serum chemerin levels were significantly reduced after surgery, indicating that chemerin might mediate metabolic alterations in obesity.

However, there have been limited studies regarding the relationship between circulating chemerin levels and atherosclerosis, the final complication of metabolic-related disorders. Only one study has evaluated the effect of serum chemerin levels on coronary atherosclerosis measured by multi-slice computed tomography (CT) angiography\(^7\). In that study, chemerin levels were weakly correlated with the coronary plaque burden \(r = 0.16, p = 0.006\), although the association disappeared after adjusting for other cardiovascular risk factors. Multi-slice CT angiography is limited in that it can only detect advanced atherosclerotic lesions and provide no information about the functional defect\(^8\).

Therefore, in the present study we examined the relationship between the circulating chemerin levels and arterial stiffness, as represented by the brachial ankle pulse wave velocity (baPWV), which assesses structural and functional defects of the arterial wall, and the carotid intima-media thickness (IMT) in obese and non-obese subjects.

**Methods**

**Study Design and Participants**

We enrolled 120 apparently healthy participants who underwent a medical checkup in the Department of Endocrinology and Metabolism of Korea University Guro Hospital. Participants included 58 obese subjects (BMI \(\geq 25\) kg/m\(^2\)) and 62 non-obese controls (BMI < 25 kg/m\(^2\)). Obesity was defined using the BMI according to the Asia-Pacific criteria (APC-BMI: \(\geq 25\) kg/m\(^2\))\(^9\). No participants had a history of cardiovascular diseases (myocardial infarction, unstable angina, stroke, peripheral artery disease, or cardiovascular revascularization), stage 2 hypertension (resting blood pressure \(\geq 160/100\) mmHg), malignancy, or severe renal or hepatic disease. We excluded patients with a history of inflammatory conditions or patients taking medications that might affect vascular function, such as lipid-lowering agents, anti-hypertensive drugs, anti-platelet agents, estrogen, vasodilators, antioxidant vitamin supplements, insulin, thiazolidinedione, and non-steroidal anti-inflammatory drugs, within the previous 6 months. We also excluded smokers from our study because cigarette smoking may influence vascular stiffness. All participants provided written informed consent, and the Korea University Institutional Review Board, in accordance with the Declaration of Helsinki of the World Medical Association, approved this study protocol.

**Clinical and Laboratory Measurements**

The BMI was calculated as the weight/height\(^2\) (kg/m\(^2\)) and the waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest. All blood samples were obtained in the morning after a 12-hour overnight fast, and were immediately stored at \(-80^\circ\)C for subsequent assays. Serum triglycerides and high density lipoprotein (HDL)-cholesterol were determined enzymatically using a chemistry analyzer (Hitachi 747; Hitachi Inc., Tokyo, Japan). The low-density lipoprotein (LDL)-cholesterol concentration was estimated using the Friedewald formula. The glucose oxidase method was used to measure plasma glucose and an electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN, USA) was used to measure insulin levels. Insulin resistance was calculated by the homeostasis model assessment of insulin resistance (HOMA-IR). Metabolic syndrome was defined according to the criteria established by the National Cholesterol Education Program Adult Treatment Panel III using the adjusted waist circumference for Asians\(^10\). Serum concentrations of chemerin were determined by an enzyme-linked immunosorbent assay (ELISA) kit (Biovender Laboratory Medicine Inc., Modrice, Czech Republic); the intra- and inter-assay variations were 6.1% and 7.6%, respectively.

**Measurement of PWV**

After a subject had rested in the supine position for 5 min, the baPWV was measured using a volume-plethysmographic apparatus (model BP-203RPE II;
Colin, Komaki, Japan), which simultaneously records the baPWV, and the brachial and ankle blood pressures on the left and right sides. Details of this method, including the validity and reproducibility, have been described previously by Yamashina et al.\textsuperscript{11}. The intra- and inter-observer reproducibility of this method was 10.0% and 8.4%, respectively. In the current study, the baPWV was calculated as the mean of the left and right baPWV values.

### Measurement of Carotid IMT

The IMT of the common carotid artery was determined using high-resolution B-mode ultrasonography (EnVisor; Philips Medical Systems, Andover, MA, USA) with a 5-12 MHz transducer. Carotid IMT was measured using IMT measurement software (Intimascope; Media Cross Co., Tokyo, Japan) at 3 levels of the lateral and medial walls of the carotid artery, 1-3 cm proximal to the carotid bifurcation. The IMT was the average value of 99 computer-based points in the region. In the present study, the carotid IMT was calculated as the mean of the left and right IMT value. All measurements were recorded by one trained technician who was blinded to the subject’s anthropometric and laboratory data.

### Statistical Analysis

Each variable was assessed for a normal distribution. Data are expressed as the mean ± SD or median (inter-quartile range [25%-75%]). Differences between groups were tested using an independent two-sample \textit{t}-test or the Mann-Whitney \textit{U}-test for continuous variables, and the chi-square test was used to test for differences in the distribution of categorical variables. Pearson’s correlation test was performed to determine the relationships between serum chemerin levels and study variables, including the baPWV and mean carotid IMT values. Before correlation analysis, a natural logarithmic transformation was performed for abnormally distributed variables. The association between the baPWV and each risk factor was assessed by dividing the patients into tertiles on the basis of the baPWV, and analyzed with ANOVA for normally distributed variables and the Kruskal-Wallis \textit{H} test for skewed variables. Subsequent pair-wise comparisons were performed using Tukey’s HSD post-hoc analysis or the Wilcoxon rank-sum test. Multiple linear step-wise regression analysis with baPWV as a dependent variable was performed to identify the risk factors which determined the baPWV. All statistical results were based on two-sided tests. Data were analyzed using SPSS for Windows (version 12.0; SPSS Inc., Chicago, IL, USA). \textit{P} < 0.05 was regarded as significant.

<table>
<thead>
<tr>
<th>Table 1. Clinical and laboratory characteristics of the study subjects</th>
<th>Non-obese group ((n = 62))</th>
<th>Obese group ((n = 58))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.63 ± 7.06</td>
<td>53.47 ± 9.14</td>
<td>0.574</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>27/35</td>
<td>25/33</td>
<td>0.961</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>22.65 ± 1.68</td>
<td>27.23 ± 1.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>85.98 ± 8.15</td>
<td>93.91 ± 7.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>120.15 ± 13.32</td>
<td>124.82 ± 16.57</td>
<td>0.092</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>76.37 ± 9.67</td>
<td>84.4 ± 9.90</td>
<td>0.008</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.78 ± 0.84</td>
<td>4.56 ± 0.75</td>
<td>0.768</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.06 (1.15, 2.83)</td>
<td>2.86 (1.87, 3.98)</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.36 ± 0.62</td>
<td>2.5 ± 0.76</td>
<td>0.187</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.35 ± 0.35</td>
<td>1.3 ± 0.33</td>
<td>0.418</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.11 (0.87, 1.49)</td>
<td>1.45 (1.02, 1.96)</td>
<td>0.018</td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>0.58 (0.31, 1.28)</td>
<td>0.9 (0.48, 3.38)</td>
<td>0.006</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.74 ± 0.15</td>
<td>0.78 ± 0.21</td>
<td>0.218</td>
</tr>
<tr>
<td>Mean carotid IMT (mm)</td>
<td>0.71 ± 0.11</td>
<td>0.74 ± 0.13</td>
<td>0.253</td>
</tr>
<tr>
<td>baPWV (m/sec)</td>
<td>12.98 ± 1.23</td>
<td>14.53 ± 2.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chemerin (ng/mL)</td>
<td>0.86 ± 0.23</td>
<td>0.19 ± 0.24</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± standard deviation or median [inter-quartile range]; \(p\)-values were calculated by an independent two-sample \textit{t}-test or the Mann-Whitney \textit{U}-test was applied. BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; IMT, intima-media thickness; PWV, pulse wave velocity.
Results

Characteristics of Study Subjects

The clinical and biochemical characteristics of the study subjects are presented in Table 1. The obese group (BMI ≥25 kg/m²) had increased waist circumference, diastolic blood pressure, HOMA-IR, triglycerides, and high sensitivity C-reactive protein (hsCRP) compared with the lower BMI group (<25 kg/m²). Furthermore, the baPWV values were significantly higher in the obese group than in the non-obese group, whereas there was no significant difference in the mean carotid IMT values between the two groups. The circulating chemerin level in the obese group was significantly higher than in the non-obese group (120.14 ± 19.34 vs. 106.81 ± 23.39, p < 0.01; Table 1).

Correlation Between Serum Chemerin Levels and Study Variables, Including baPWV and Mean Carotid IMT

The circulating chemerin level had a significant positive correlation with BMI (p < 0.001), waist circumference (p < 0.001), HOMA-IR (p = 0.001), and LDL-cholesterol (p = 0.042), triglycerides (p < 0.001), and hsCRP (p = 0.004) levels. Furthermore, the serum chemerin level was positively associated with the number of metabolic syndrome components (p = 0.006; Table 2). Importantly, the serum chemerin level had a positive correlation with the baPWV (r = 0.280, p = 0.002), but not with the mean carotid IMT (r = 0.065, p = 0.504; Fig. 1).

Clinical and Laboratory Variables Stratified by Tertiles of the baPWV

Based on the increments of the baPWV values, age, BMI, waist circumference, systolic blood pressure, fasting glucose, HOMA-IR, serum hsCRP level and the number of metabolic syndrome components were increased. Specifically, the circulating chemerin levels exhibited a significant increment according to the elevation of the baPWV; however, the diastolic blood pressure, LDL-cholesterol, HDL-cholesterol, and triglycerides had no significant trends with increments in the baPWV levels (Table 3).

Factors Determining the baPWV

Multiple stepwise regression analysis showed that age (p < 0.001), waist circumference (p = 0.038), systolic blood pressure (p < 0.001), and serum fasting glucose (p = 0.003) and chemerin levels (p = 0.017) were definitive risk factors for arterial stiffness (r² = 0.457; Table 4).

Table 2. Pearson’s correlation analysis between serum chemerin levels and study variables, including mean carotid IMT and baPWV

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.098</td>
<td>0.288</td>
</tr>
<tr>
<td>BMI</td>
<td>0.327</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.352</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>−0.024</td>
<td>0.798</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>−0.028</td>
<td>0.761</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.001</td>
<td>0.992</td>
</tr>
<tr>
<td>HOMA-IR*</td>
<td>0.310</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.186</td>
<td>0.042</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>−0.032</td>
<td>0.733</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>0.382</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsCRP*</td>
<td>0.262</td>
<td>0.004</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.132</td>
<td>0.153</td>
</tr>
<tr>
<td>No. of MetS components</td>
<td>0.254</td>
<td>0.006</td>
</tr>
<tr>
<td>Mean carotid IMT</td>
<td>0.065</td>
<td>0.504</td>
</tr>
<tr>
<td>baPWV</td>
<td>0.280</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Logarithmic transformed data were used.

BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; MetS, metabolic syndrome; IMT, intima-media thickness; baPWV, brachial ankle pulse wave velocity.

Discussion

The present study confirmed that the serum chemerin level is elevated in obese compared with lean individuals and has significant positive correlations with waist circumference, diastolic blood pressure, HOMA-IR, and serum triglycerides and hsCRP levels. Most importantly, we demonstrated for the first time that the circulating chemerin level was positively associated with arterial stiffness, as represented by the baPWV, and the serum chemerin level was an independent determining factor for the baPWV, even after adjusting for other cardiovascular risk factors.

The prevalence of obesity is increasing markedly, especially in children and adolescents. Many studies have demonstrated that obesity increases mortality from all causes, including cardiovascular deaths. There are some plausible mechanisms by which obesity can adversely affect vascular health. First, obesity aggravates blood pressure, the glucose level, and lipid metabolism, and thereby indirectly increases cardiovascular risk. In addition, growing evidence has shown that adipokines, which are secreted by adipose tissue, play a direct essential role in the formation of atherosclerotic lesions. For example, resistin, one of the well-known adipokines, directly activates the endothelium through up-regulation of adhesion molecules, and also
Table 3. Clinical and laboratory variables stratified by tertiles of baPWV

<table>
<thead>
<tr>
<th></th>
<th>1st quartile (n=39)</th>
<th>2nd quartile (n=38)</th>
<th>3rd quartile (n=41)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>baPWV (m/sec)</td>
<td>(9.77-12.67)</td>
<td>(12.67-14.17)</td>
<td>(14.17-23.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.87 ± 6.04</td>
<td>51.82 ± 7.58</td>
<td>58.33 ± 7.33</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.63 ± 2.83</td>
<td>24.89 ± 2.30</td>
<td>25.97 ± 3.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>84.11 ± 8.76</td>
<td>92.22 ± 6.53</td>
<td>92.67 ± 8.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>117.92 ± 12.68</td>
<td>120.81 ± 13.52</td>
<td>127.8 ± 17.14</td>
<td>0.010</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>78.49 ± 14.21</td>
<td>77.81 ± 9.79</td>
<td>80.61 ± 11.52</td>
<td>0.559</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.77 ± 0.92</td>
<td>6.75 ± 1.72</td>
<td>6.57 ± 1.85</td>
<td>0.014</td>
</tr>
<tr>
<td>HOMA-IR*</td>
<td>2.08 (0.96, 2.81)</td>
<td>2.62 (1.92, 4.73)</td>
<td>2.38 (1.67, 3.51)</td>
<td>0.020</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.32 ± 0.68</td>
<td>2.39 ± 0.66</td>
<td>2.53 ± 0.73</td>
<td>0.120</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.34 ± 0.30</td>
<td>1.23 ± 0.32</td>
<td>1.39 ± 0.39</td>
<td>0.128</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)*</td>
<td>1.12 (0.71, 1.79)</td>
<td>1.22 (0.88, 1.92)</td>
<td>1.49 (1.02, 1.95)</td>
<td>0.182</td>
</tr>
<tr>
<td>hsCRP (mg/dL)*</td>
<td>0.53 (0.32, 0.97)</td>
<td>0.82 (0.41, 3.73)</td>
<td>1.00 (0.46, 2.59)</td>
<td>0.046</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.76 ± 0.17</td>
<td>0.76 ± 0.18</td>
<td>0.77 ± 0.20</td>
<td>0.986</td>
</tr>
<tr>
<td>Mean carotid IMT (mm)</td>
<td>0.70 ± 0.13</td>
<td>0.71 ± 0.10</td>
<td>0.75 ± 0.12</td>
<td>0.262</td>
</tr>
<tr>
<td>Chemerin (ng/mL)</td>
<td>103.64 ± 19.92</td>
<td>115.23 ± 24.78</td>
<td>119.18 ± 18.76</td>
<td>0.004</td>
</tr>
<tr>
<td>Number of MetS components</td>
<td>2 (1.00, 3.00)</td>
<td>3 (2.00, 3.00)</td>
<td>3 (2.00, 3.35)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD or median (inter-quartile range).

p-value for overall difference among the groups was calculated using analysis of variance (ANOVA) or the Kruskal-Wallis H test.*

a, b, c: Same letter indicates no statistical difference based on Tukey’s HSD post-hoc analysis or Wilcoxon’s rank sum test.

baPWV, brachial ankle pulse wave velocity; BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; IMT, intima-media thickness; MetS, metabolic syndrome.

Fig. 1. Correlation between circulating chemerin levels and mean carotid IMT (A), and baPWV (B).
Table 4. Multiple stepwise regression analyses for factors associated with baPWV

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.089</td>
<td>0.019</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.043</td>
<td>0.021</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.046</td>
<td>0.010</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.017</td>
<td>0.006</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Chemerin</td>
<td>0.018</td>
<td>0.008</td>
<td>0.017</td>
<td>0.457</td>
</tr>
</tbody>
</table>

Independent variables in multiple stepwise regression analysis: gender, age, body mass index, waist circumference, systolic blood pressure, diastolic blood pressure, fasting blood glucose, HDL-cholesterol, LDL-cholesterol, triglycerides, high sensitivity C-reactive protein, and chemerin
baPWV, brachial ankle pulse wave velocity; SE, standard error; R², coefficient of determination; BP, blood pressure.

promotes the migratory activity of vascular smooth muscle cells[13]. Furthermore, Fu et al.[14] demonstrated that adenovirus-mediated over-expression of adipocyte fatty acid binding protein (A-FABP) induces foam cell formation by increasing intracellular lipid accumulation in macrophage cell lines, which is an essential step in the formation of atherosclerotic plaques. Recently, we reported that serum resistin and A-FABP are independently associated with atherosclerotic inflammation, as determined by [18F]-fluorodeoxyglucose positron emission tomography[15, 16].

The chemerin gene was originally described as a retinoid-responsive gene in psoriatic skin lesions[17]. Although chemerin is a well-known secreted protein with an established role in immune function, recent experimental data indicate that chemerin might provide a link between obesity and chronic inflammation[18]. Sell et al.[19] reported that chemerin activated the NF-κB pathway and impaired glucose uptake in primary human skeletal muscle cells. Moreover, TNF-α treatment of 3T3-L1 adipocytes increased bioactive chemerin levels, suggesting that inflammatory cytokines contribute to the up-regulation of chemerin in obesity[20]. Thus, it is possible that adipocyte-derived chemerin may be involved in the pathogenesis of obesity-related inflammatory disorders, including atherosclerosis; however, very few studies have examined the influence of circulating chemerin on the atherosclerotic process. In 41 autopsy cases, coronary atherosclerosis was positively correlated with the expression of local chemerin in pericoronary fat[21]. Lehrke et al.[7] showed that circulating chemerin had a positive correlation with atherosclerotic plaque burden assessed by multi-slice CT angiography, but this association was lost after adjusting for established cardiovascular risk factors. Multi-slice CT angiography has several limitations, which include inability to document early endothelial dysfunction, preceding the morphologic changes of the arterial wall[10].

Arterial stiffness is recognized as a result of structural and functional changes of the vascular tree[22]. Vascular endothelium plays an important role in the functional regulation of arterial stiffness by secreting nitric oxide, endothelin-1, and natriuretic peptides[23]. Endothelial dysfunction is an early and potentially reversible event in atherogenesis[24]. In contrast, carotid IMT quantitatively measures the arterial morphology consisting of intimal lesions and medial hypertrophy[25]. In animal studies it has been shown that altered arterial compliance precedes angiographically detectable atherosclerosis in LDL-receptor deficient rabbits[26]. Furthermore, the Atherosclerosis Risk in Communities (ARIC) study demonstrated that there was no association between arterial wall thickness and increased arterial stiffness, except for the thickest 10% of the artery walls[27]; therefore, arterial stiffness and carotid atherosclerosis may differ in the pathologic background and be dissociated in time. In the present study, the serum chemerin level was independently associated with baPWV, even after adjusting for other cardiovascular risk factors, but not with carotid IMT. baPWV is a non-invasive method for measuring arterial stiffness in a short time without being influenced by the surgeon’s technique[28]. Until now, many studies have shown that PWV is an independent risk factor for cardiovascular disease[29]; during a 10-year follow-up period, Cruickshank et al.[30] reported that arterial stiffness independently predicted all-cause and cardiovascular mortality in 397 diabetic patients for each 1 m/s increase in PWV (hazard ratio, 1.08; 95% confidence interval, 1.03-1.14); therefore, our results suggest that circulating chemerin may directly mediate the process of cardiovascular disease.

There were some limitations to our study. First, this study enrolled a small number of a limited population; therefore, the relationship between circulating chemerin and arterial stiffness should be studied further in other ethnic groups, including larger subjects. Second, due to the drawback of the cross-sectional design, the causal relationship and underlying mechanism could not be defined in the present study. Very recently, a few experimental studies have examined the impact of chemerin on atherogenesis. Hart and Greaves[31] showed that chemerin rapidly stimulated the adhesion of macrophages to the extracellular matrix protein, fibronectin, and to the adhesion molecule, vascular cell adhesion molecule-1, suggesting that chemerin might promote the progression of atherosclerosis. Although Becker et al.[32] showed that the
expression of chemerin did not significantly alter the extent of atherosclerosis in LDL receptor knockout mice, they hypothesized that chemerin may affect early atherosclerotic plaque development and plaque morphology rather than the extent of the atherosclerotic lesion area. Furthermore, Kaur et al. demonstrated the novel presence of a G-protein coupled chemerin receptor (CMKLR1) in human endothelial cells and its significant up-regulation by pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6). Thus, the altered expression of chemerin and its receptors during an inflammatory process may cause dysregulated angiogenesis, leading to the development of cardiovascular disease.

In conclusion, the present study confirmed that the circulating chemerin level is significantly elevated in obese individuals and has a close correlation with various metabolic risk factors. Moreover, we demonstrated that the serum chemerin level was independently associated with arterial stiffness, even after adjusting for other cardiovascular risk factors. Further experimental studies are warranted to clarify the role of chemerin in the atherosclerotic process.

Acknowledgements

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References


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31) Hart R, Geaves DR: Chemerin contributes to inflammation by promoting macrophage adhesion to VCAM-1 and fibronectin through clustering of VLA-4 and VLA-5. J Immunol, 2010; 185: 3728-3739


Reviewer 1 Comments for the Author...

The authors concluded that the circulating chemerin level was an independent risk factor for arterial stiffness in obese persons, even after adjusting for other cardiovascular risk factors.

The topic seems to be new.

1) However, the major concern of this reviewer is that P-value is barely significant (p=0.048 in Table 4). And even this weak significance disappeared in Non-obese group. The authors did not appear to provide explanation for this discrepancy between Obese and Non-obese group. In contrast to circulating chemerin, age, systolic BP and fasting glucose show much stronger independent association with baPWV, which also clouds the importance of chemerin as an independent determinant of arterial stiffness.

Reply: We apologize for the mistake in the description of the focus of our study in the previous manuscript. We wanted to emphasize that the serum chemerin level might have an independent significant influence on arterial stiffness, even after adjusting for other well-known cardiovascular risk factors. The focus of our study was not the differential relationship between circulating chemerin and arterial stiffness according to the obese and non-obese groups. To clarify the importance of chemerin as an independent factor for arterial stiffness, we performed multiple stepwise linear regression analysis for all of the subjects, including both obese and non-obese individuals (n=120). As a result, age (p<0.001), waist circumference (p=0.038), systolic blood pressure (p<0.001), and the fasting blood glucose (p=0.003) and serum chemerin levels (p=0.017) were independent factors determining the baPWV. As shown in supplemental Fig. 1, the serum chemerin level did not remain an independent factor for arterial stiffness in the non-obese group by a narrow margin (p=0.053). When the sample size was expanded to all subjects (n=120, including the non-obese group) in the multiple regression model, the p-value for chemerin was 0.017. Thus, we can assume that the marginal p-value of the obese group in the previous manuscript (Table 4) was due to the small sample size (n=58). If you do not object, we would like to omit the statement that the circulating chemerin was a significant determining factor in the obese group alone in the revised manuscript and Table 4 as this was not our major point. We apologize for the ambiguity.

2) Another concern is that the sample size of this study is too small.

Reply: We agree that the small sample size was a limitation in this study. This limitation and the need for a larger clinical study have been added on page 12 in the 2nd paragraph; however, the study is still important, demonstrating for the first time the independent relationship of a novel adipokine, chemerin, with arterial stiffness.

3) In Discussion, the authors describe the limitations of multi-slice CT and carotid IMT in detecting early sign of early endothelial dysfunction, whereas baPWV is able to detect early sign of cardiovascular disease. This reviewer is wondering this is generally accepted.

Reply: Thank you for your thoughtful comments. After an exhaustive review of numerous references about the relationship between arterial stiffness (assessed by baPWV) and atherosclerotic burden (measured by MD-CT or carotid IMT), we agree that it is not clear whether artery wall stiffness precedes atherosclerosis and the baPWV necessarily assesses earlier cardiovascular disease than carotid IMT or multi-slice CT. The relationship between arterial stiffness and atherosclerosis seems to be more complicated and to affect each other rather than having a definite order (Kobayashi et al., Atherosclerosis 2004; 173: 13-8; Zureik et al., J Hypertension 2002; 20: 85-93).

We want to emphasize that multi-slice coronary CT, carotid IMT, and baPWV evaluate different aspects of atherosclerosis and chemerin might mediate the process of arterial stiffness. Thus, we revised the Discussion section about the differences in the various screening modalities for atherosclerosis and the relationships with circulating chemerin on page 11-12 and the 2nd-1st paragraph.

We appreciate your in-depth review of our manuscript. We hope that our responses satisfy your thoughtful and helpful comments.

Reviewer 2 Comments for the Author...

This study is well designed and provides an important information. There are some minor points.

1) In Abstract and Results sections, the serum chemerin level was significantly increased in obese...(120 vs. 106).

Reply: Yes, we changed the order of the numbers from 106.81 ± 23.39 vs. 120.14 ± 19.34 to 120.14 ± 19.34 vs. 106.81 ± 23.39.

2) angiogenesis in Page 12

Reply: We corrected the spelling error from angiogenesis to angiogenesis.

3) Was serum chemerin level different between men and women because it is strongly expressed in white adipose tissue?
Reply: No, there was no significant difference between the circulating chemerin levels in men and women (111.00 ± 19.92 vs. 114.97 ± 24.22, p = 0.34).

In agreement with our study, Stejskal et al. (Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2008; 152: 217-221) showed that chemerin had no gender dimorphism in type 2 diabetic patients and in a cohort of healthy volunteers.

Thank you for your kind comments.

Reviewer 3 Comments for the Author...

In this paper the authors investigated the relationship between circulating chemerin level and arterial stiffness in obese persons. The article is interesting and well written.

Minor comments/questions:

1) The discussion should be improved. Chemerin related sentences written in the introduction were repeated in the discussion.

Reply: As per your recommendation, we shortened the repeated sections in the Discussion section and supplemented the required content according to the reviewer’s comments.

2) Also, the authors should mention that one of the limitations of the study is the small sample size.

Reply: We have added this limitation in the Discussion section (page 12, 2nd paragraph).

3) In the methods, p5, replace “HDL-C” with “high density lipoprotein (HDL)-cholesterol”.

Reply: This has been corrected.

4) In the results, p7, it should be instead “(BMI ≥ 25 kg/m²)” “(BMI > 25 kg/m²)”

Reply: This has been changed.

5) In the results, p7, replace “(BMI < 25 kg/m²)” group with “(BMI < 25 kg/m²)”

Reply: This has been changed.

Thank you for your useful comments.