CHEMERIN BLOOD LEVELS ARE ASSOCIATED WITH MRI MEASURED VOLUMES OF ABDOMINAL ADIPOSE TISSUE COMPARTMENTS AND LIFESTYLE CHOICES

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SUMMARY – Obesity is a low-grade chronic inflammatory state, in which a cytokine chemerin with its immunometabolic modulation has an important role. We aimed to study in a healthy population relationships between serum chemerin levels, lifestyle choices and magnetic resonance imaging (MRI) assessed volumes of abdominal visceral (VAT) and subcutaneous (SAT) adipose tissues, which have different cytokine expression profiles. Overall, 73 healthy participants undertook lifestyle questionnaire and underwent anthropometric measurements along with MRI scanning of abdominal SAT and VAT. Furthermore, complete blood count was determined along with chemerin and fibrinogen serum levels. Regression model for prediction of chemerin serum levels was built after controlling for sex, age and anthropometric measures. Median serum chemerin was 141 (125-195) ng/mL. Serum chemerin had a moderate positive correlation with SAT and VAT volumes. The two most important predictors of chemerin levels were MRI detected SAT and thigh circumference. Independently, chemerin levels were associated with smoking, preference of salty food, and favoring flavor/simplicity of preparation over nutritional value of the food. Serum chemerin seems to be more strongly correlated with the volume of abdominal SAT than VAT, with certain lifestyle choices influencing chemerin levels independently of abdominal fat.

Key words: Obesity; Cytokines – blood; Magnetic resonance imaging; Fibrinogen; Abdominal fat; Adipokines; Intra-abdominal fat; Lifestyle; Smoking

Introduction

Obesity is recognized as a low-grade chronic inflammatory state. It is associated with increased mortality and numerous morbidities including metabolic syndrome, diabetes mellitus type 2 and coronary artery disease1,2. Adipose tissue (AT) secretes immunometabolic modulators called adipokines. Excessive AT has altered secretion profile, where several adipokines (or their lack) contribute to the pathophysiology of these comorbid diseases3,4. One such molecule secreted by AT is chemerin, also known as retinoic acid receptor responder protein 2 (RARRES2)5. As a potent signaling molecule, chemerin has been described as adipokine, cytokine, paracrine/autocrine hormone, tu-
CMLKR1 is expressed in various tissues, with highest expression in several inflammatory (spleen, placenta and lymph nodes) and non-inflammatory related sites (vena cava, lungs and urinary bladder)\(^7,8\). CMLKR1 expression profile is notably high in dendritic cells and macrophages\(^7,8\).

Chemerin regulates lipid metabolism and mesenchymal stem cell adipogenesis through autocrine/paracrine mechanisms\(^11,12\). Lack of chemerin or CMLKR1 expression in vitro abrogates adipogenesis, and modifies the expression of genes important in glucose and lipid metabolism, including glucose transporter type 4 (GLUT4), leptin and adiponectin\(^13\). Higher chemerin expression in AT is associated with metabolic syndrome and its metabolic disturbances\(^14\). Furthermore, chemerin mediates infiltration of macrophages in AT, and these are considered to be crucial cells in inflammatory activation of adipocytes in obesity\(^13,15\).

Morbidity/mortality risk is higher for abdominal than for overall obesity. Overall abdominal fat is a sum of subcutaneous (SAT) and visceral (VAT) adipose tissue, the latter consisting mainly of mesenteric, omental and retroperitoneal fat tissue\(^16\). Ibrahim et al.\(^17\) have shown that, in comparison to SAT, VAT contains higher amounts of inflammatory immune cells and greater number of large adipocytes, and that its pre-adipocytes have a weaker capacity to differentiate. Furthermore, VAT adipocytes are more insulin resistant, more metabolically active, and secrete a larger number of cytokines. Correlation of metabolic risk factors (increased body mass index (BMI), triacylglyceride, glucose, blood pressure, C-reactive protein (CRP) and decreased high density lipoprotein) with SAT and VAT has already been established, with the latter showing stronger correlation\(^18\). Furthermore, VAT correlates to a greater extent with metabolic risk factors in women\(^19\). Alfadda et al.\(^19\) in a study of overweight subjects (BMI \(>25\) kg/m\(^2\)) did not establish difference in serum chemerin level between metabolically healthy (had fewer than two above mentioned metabolic risk factors) and metabolically unhealthy obese.

Chemerin mRNA expression in VAT and SAT in lean individuals was significantly lower than in obese patients, but did not differ in the expression levels between VAT and SAT\(^20\). This suggested that correlation between chemerin and BMI was only due to the increase in fat mass. On the contrary, mRNA chemerin expression in patients with type 2 diabetes mellitus and obese subjects was higher in VAT than in SAT\(^9\). A recent large study by Zyilla et al.\(^21\) established the association between AT volumes, inflammation markers and chemerin levels.

As distribution of AT is of a well-known importance (i.e. for cardiovascular disease), there are numerous attempts to directly, through imaging techniques, or indirectly (i.e. using different anthropometric measures) assess the quantity of AT in different depots. Assessment of SAT and VAT by magnetic resonance imaging (MRI) or computed tomography (CT) and post mortem assessment correlated well\(^22\). Anthropometric measures showed positive correlations with post mortem AT volumes only in certain study groups (i.e. quantity of VAT measured by MRI (20 cm above and 10 cm below the L4 and L5 intervertebral disk) in obese subjects (BMI 30.0 to 39.9 kg/m\(^2\)), and positive correlation with waist circumference (WC) and waist-hip ratio (WHR) only in females\(^23\).

Only a few studies have investigated the relationship between AT volumes and blood chemerin\(^21,24\). Metabolic disturbances do not appear only among obese individuals (i.e. normal-weight obesity syndrome), and the knowledge about the underlying molecular and environmental mechanisms is rather scarce. Due to the association of chemerin with metabolic disturbances\(^13,15\), it would be of interest to get an insight into the effect of environmental factors (i.e. diet, physical activity) on chemerin level and body fat distribution in a healthy, non-obese population. Moreover, it would be valuable to study long-term effects of these ‘lifestyle’ factors. In order to answer some of these questions, we studied relationships between chemerin, dietary/lifestyle habits and volumes of abdominal VAT and SAT in a cross-sectional manner in a healthy, non-obese population.
The goals of this study were to establish (1) relationship between chemerin serum levels and adipose tissue volumes of abdominal region; (2) to compare anthropometric measures to MRI acquired parameters in chemerin serum level prediction; and (3) to explore how certain lifestyle and dietary habits influence chemerin serum levels in a healthy, adult population.

Patients and Methods

Physical examination, laboratory blood tests and health/habits/lifestyle questionnaire

The study was approved by the local ethics committee and all persons gave their informed consent prior to their inclusion in the study. The sample consisted of 73 healthy Caucasians (34 females), recruited volunteers and students from the School of Medicine, University of Osijek, Croatia. Medical history was taken in each subject and only those without prior medical conditions were included in the study. The median age of the participants was 24 (21-33) years. Physical examination included anthropometry (weight, height, WC at the level of umbilicus, hip circumference at the level of greater trochanters, thigh circumference at the middle third of the thigh, triceps skinfold thickness at the middle third of the upper arm, and subscapular skinfold thickness (SST)), vital signs check (blood pressure), and laboratory blood tests (complete blood count, fibrinogen, chemerin). All participants undertook a questionnaire assessing lifestyle and eating habits. The mean arterial pressure (MAP) was calculated as the sum of diastolic blood pressure and one-third of systolic blood pressure. VAT/SAT is the ratio of abdominal VAT volume to SAT volume. BMI was defined as the ratio of weight in kilograms to squared height in meters.

MRI

The SAT and VAT were measured with MRI (Siemens Magnet Avanto 1.5T MRI System, DMS Health Technologies, ND, USA), using standardized T1 ‘fast spin echo’ sequence for measuring AT. The abdominal region of interest was from the upper liver edge to the pubic symphysis. AT volume calculation was based on approximately 20 slices per participant. The program used for analyzing MRI images was open-source ImageJ. Adipose tissue was distinguished using intensity threshold macro-program, which made pictures binary, leaving only those intensities above curtain cut off value. On each slab, surface area of SAT and VAT was manually measured using the region of interest principal. The volume of AT was calculated according to the MRI parameters, multiplying surface area of adipose tissue on each slab with slab thickness and corresponding distance between the measured slab and subsequent one. MRI acquisition and post processing was done by a radiologist with more than 10 years of experience in MRI body imaging.

Chemerin ELISA

Chemerin was quantified using human chemerin enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences, Farmingdale, NY, USA). Blood sampling was performed early in the morning on an empty stomach, after less than 12-hour fasting.

Statistics

Values are presented as median [Q1-Q3] or mean (±SD) depending on the distribution. Distribution of variables was tested using Shapiro-Wilk test. Normally distributed variables were compared using parametric Student’s t-test and one-way ANOVA. Variables that were not normally distributed were compared using nonparametric Mann-Whitney and Kruskal-Wallis tests. In order to perform relationship analyses, Pearson correlation and multiple regression analysis with forward stepwise method of fitting were used. Prior to regression model building, independent predictor variables were screened for multicolinearity and singularity. A two-sided p<0.05 was considered significant. IBM SPSS Statistica v. 15.0 was used on statistical analyses.

Results

The overall descriptive results of the study participants are shown in Table 1. Chemerin blood levels correlated with VAT and SAT volume (r=0.419, p<0.001; and r=0.482, p<0.001, respectively) (Fig. 1B and 1C), subscapular skinfold thickness and WC (r=0.364, p=0.009, and r=0.289, p=0.036, respectively). Other classical anthropometric measures did not correlate (data not shown). Among analyzed blood parameters, chemerin blood levels showed a moderately
strong positive correlation with fibrinogen levels (r=0.403, p=0.004), whereas mean corpuscular volume (MCV) showed a weak negative correlation (r=-0.284, p=0.036), and other parameters did not correlate with chemerin. Fibrinogen blood levels had a weak positive correlation with SAT volume (p=0.047), but not with VAT volume (p=0.074) (Fig. 1E and 1F).

To predict chemerin serum levels, a stepwise regression model was employed with MRI measured adipose tissue volumes, anthropometric measures, complete blood count and fibrinogen levels as independent predictors controlled for age and sex. The final model explained 40.6% of variance in chemerin serum levels and included the following variables as the most important predictors (from most to least important): thigh circumference, SAT, fibrinogen, hip circumference, and red blood cells (RBC), while other independent variables were excluded from the final model (Table 2).

### Lifestyle and dietary habits

Participants that preferred salty food had higher chemerin (163±46 vs. 136±40 ng/mL, p=0.045) and fibrinogen (3.15±0.66 vs. 2.85±0.40 mg/dL, p=0.05) levels. The subjects considering nutritional facts to be the most important factor when buying food had lower chemerin than those who found flavor (p=0.029) and simplicity of preparation (p=0.005) to be most important. Study participants that smoked (37.3%) had higher chemerin serum levels compared to those who...
BMI (A), as well as other classical anthropometric measures, except for waist circumference and subscapular skinfold thickness, did not correlate with serum chemerin. MRI measured volumes of abdominal visceral (B) and subcutaneous (C) adipose tissue had a moderate positive correlation with serum chemerin. Chemerin and blood fibrinogen levels (D) showed moderately strong positive correlation. Fibrinogen levels did not correlate with abdominal visceral adipose tissue volume (E), but it showed a weak positive correlation with abdominal subcutaneous adipose tissue volume (F).

BMI = body mass index; VAT = visceral adipose tissue; SAT = subcutaneous adipose tissue
Participants who consumed alcohol on a regular basis (87%; at least 3 times or more a month) had similar chemerin serum levels as their counterparts who did not consume alcohol (p=0.424). Additional stratification of participants by alcohol consumption rate did not show any differences in chemerin serum levels. Furthermore, study participants that exhibited binge drinking behavior (31.3%) did not differ in chemerin serum levels (149±42 vs. 175±55 ng/mL, p=0.123).

Furthermore, those who considered nutritional facts most important had less VAT (p=0.048) and SAT (p=0.040) than those who considered price as the main criterion whether to purchase the product. Participants who had breakfast more often (6-7 times a week) had less SAT compared to those who had breakfast less often (3-5 times a week) or not at all (p=0.018 and p=0.028, respectively). There was also a difference between the groups with different breakfast habits in hip circumference and thigh circumference (p=0.058 and p=0.032, respectively). Also, participants who frequently ate small meals (snacks) several times a week or every day, had smaller WC (p=0.037 and p=0.021, respectively) and thigh circumference (p=0.009 and p=0.053, respectively) than those who snacked seldom or never.

Participants who preferred high-fat dairy products had a higher VAT/SAT ratio than those taking medium- or low-fat dairy products (p=0.040 and p=0.020, respectively). People who were choosing food for themselves had lower thigh circumference than those whose food was chosen by their partner or family (p=0.036 and p=0.013, respectively). Participants consuming olive oil regularly (compared to those using it occasionally or never) had greater triceps skinfold thickness (p=0.001 and p=0.004, respectively) and SST (p=0.019 and p=0.003, respectively). People consuming chocolate more than once a week had lower mean arterial pressure than people enjoying chocolate 1-4 times per month or less than once a month (p=0.040 and p=0.004, respectively).

**Discussion**

Chemerin is suggested as a biomarker of visceral adiposity and associated inflammatory process. A recent study by Zylla et al. was the first to use MRI to assess volumes of total abdominal AT and chemerin. In line with our results, they established that both SAT and VAT were positively, mutually independently, associated with chemerin. Due to the suggested role of chemerin as a predictor and risk factor for the development of metabolic syndrome and atherosclerotic process, it would be valuable to identify individuals at risk through low-cost measures such as classical anthropometric measures. Similar to our results, Zylla et al. clearly showed that chemerin increased steadily with increasing WC. Other inexpensive anthropometric measures such as triceps skinfold thickness and SST might also be of interest. Herein, we presented correlation of chemerin with SST that has not been established before. SST is a seldom used anthropometric parameter and is usually considered as a measure of truncal SAT. Interestingly, SST was found to be the only valuable classical anthropometric measure which distinguished between transient and persistent

<table>
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<th>Independent predictor</th>
<th>Coefficient</th>
<th>p</th>
<th>95% Confidence interval</th>
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<tr>
<td>Thigh circumference (cm)</td>
<td>-7.256</td>
<td>0.001</td>
<td>-11.241</td>
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<tr>
<td>Subcutaneous adipose tissue (mL)</td>
<td>0.011</td>
<td>0.001</td>
<td>0.005</td>
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<td>Fibrinogen (g/L)</td>
<td>31.284</td>
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<tr>
<td>Hip circumference (cm)</td>
<td>2.340</td>
<td>0.028</td>
<td>0.269</td>
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<td>Red blood cells (x10^12/L)</td>
<td>23.428</td>
<td>0.044</td>
<td>0.709</td>
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<tr>
<td>Intercept</td>
<td>-159.536</td>
<td>0.248</td>
<td>-433.408</td>
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impaired glucose tolerance. Also, Savastano et al. showed that SST was the adiposity measure with the strongest negative association with blood level of insulin-like growth factor-1 binding protein 1, known to be altered inversely proportionally to the degree of visceral adiposity. On the contrary, a study by Vasan et al. implied that abdominal skinfold rather than SST was associated with elevated blood glucose. Nevertheless, it would be interesting to include SST in future studies aimed to assess factor clustering in metabolic and atherosclerotic phenotypes (i.e. disturbances in glucose, insulin and blood lipid regulation).

Not surprisingly, chemerin showed stronger correlation with MRI-assessed volumes of SAT and VAT than with the traditional anthropometric measures. On the other hand, regression analysis showed that, besides SAT, a ‘classical’ thigh circumference (a measure of SAT) was the most important predictor of chemerin level. A potential explanation why VAT did not emerge as a predictor of chemerin level is a modest sample size and rigorous controlling in the forward stepwise regression model. Additionally, although chemerin was clearly associated with visceral obese phenotypes, expression of chemerin mRNA in SAT and VAT did not differ in healthy, lean (BMI <25 kg/m²) adults. It is important to stress that absolute body volume of SAT is greater in both sexes. With common single L4/L5 slice approach the results are skewed and miss much of the diversity of human body fat distribution. Although we assessed the majority of body VAT, unembraced SAT is also hormonally active tissue, where classical anthropometric measures ensure an insight into its quantity in other body regions.

Solely reducing BMI after bariatric surgery showed reduction of chemerin in a follow-up study. Alternatively, after a 12-week exercise program, chemerin levels decreased without change in BMI. The exercise did, however, increase insulin sensitivity and decreased inflammatory activity (as assessed by CRP). Several studies also showed that blood chemerin decreased after exercise and dietary interventions (calorie restriction and education), but did not investigate separately the effects of dietary habits. An interesting study by Khoo et al. on obese men after similar daily negative energy balance for 24 weeks, either through exercise regimen or through diet-intervention, showed similar decrease in weight and WC, but greater reduction of fat mass, chemerin and CRP in the exercise group. Additionally, only exercising participants showed increased levels of the ‘good’ adipokine, adiponectin. We showed that higher chemerin levels were present in healthy subjects who preferred salty food and those who primarily were thinking of simplicity of preparation or taste while buying food, as opposed to subjects whose primary concern was nutritive value regardless of their BMI. Such association between chemerin and dietary habits has not been documented earlier and increased sodium intake is associated with several morbidities. Although all our subjects were healthy, these data fit into the ‘healthy lifestyle picture’ of the population with lower chemerin. It is alluring to hypothesize that long-term effects of different adverse environmental factors might be partially mediated through chemerin, i.e. through long-term, silent ‘inflammation excess’.

Obesity is characterized by increased levels of chemerin and fibrinogen. Our study confirmed the association between chemerin and fibrinogen levels in a healthy population. We still lack an explanation for the association, but we can conjecture that chronic inflammation in hypertrophic AT might underlie increases in the blood levels of both parameters. Nevertheless, as both fibrinogen and chemerin have been established as independent factors associated with the development of atherosclerosis, even in healthy subjects, the possible risk of developing cardiovascular diseases should be noted. The study by Chakaroun et al. offered a valuable insight into chemerin production; mRNA expression in omental (VAT) and SAT was similar in both depots in lean and obese (but higher in obese), and higher in VAT than in SAT of diabetic subjects. Therefore, both AT depots are important producers of chemerin. Furthermore, it should be kept in mind that AT is not a single-cell type tissue and different expression profiles of cells within AT might explain discrepancies in the results from mRNA-based studies of VAT and SAT.

It should not be forgotten that chemerin is not produced only in AT; the pancreas, liver and lungs are suggested to be greater sources. Interestingly, in mice, chemerin was directly involved in local inflammatory response following exposure to cigarette smoke. Information on environmental effects on chemerin levels is rather scant. Boyuk et al. report that chronic obstructive pulmonary disease patients with a history of smoking had higher chemerin than control (never-
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smokers) group. Herenius et al. established 'a trend towards higher levels of chemerin in current smokers than in non-smokers' among patients with rheumatoid arthritis. We showed that even in healthy population chemerin was higher among smokers. These results fit into the paradigm of unhealthy lifestyle choices leading to atherosclerosis and cardiovascular disease.

Data on the effects of alcohol intake on chemerin are limited. Chronic heavy drinking (>48 g/d) increased chemerin, whereas a study on rats suggested that elevated chemerin mostly reflected increased production in hypertrophic VAT. In line with this study, in our group of mostly non-drinkers and occasional alcohol consumers, we did not establish any such association.

In conclusion, adipose tissue volumes of abdominal region moderately positively correlated with serum chemerin levels, while SAT volume, along with thigh circumference (anthropometric marker of SAT) was a more important predictor of serum chemerin levels than VAT after controlling for gender, age and BMI. Additionally, hip circumference was also shown to be an important predictor of serum chemerin levels. Furthermore, our results imply that dietary habits and lifestyle choices such as smoking, salt preference, healthy and 'slow' food, may influence blood chemerin levels independently of AT volumes. Finally, this study points in the direction of abdominal SAT and its relative contribution compared to VAT, as one of the major sources of chemerin, which might be a piece of puzzle underlying normal-weight obesity and metabolic syndrome.

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Sažetak

RAZINA KEMERINA U KRVI JE POVEZANA S VOLUMENIMA TRBUŠNOG MASNOG TKIVA IZMJERENIMA POMOĆU MAGNETSKE REZONANCIJE I ČIMBENICIMA STILA ŽIVOTA

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Pretilost je stanje obilježeno kroničnom upalom niskog intenziteta u kojoj citokin kemerin, imunometabolički modulator, ima važnu ulogu. Cilj studije bio je istražiti odnose između serumskih razina kemerina, životnih navika i volumena abdominalnog visceralnog (VMT) i subkutanog (SMT) masnog tkiva. U studiji su bila istražena 73 zdrave sudionike kojima je u traženju odgovoran upitnik o životnim navikama, potom su utvrđeni volumeni abdominalne i subkutanog masnog tkiva pomoću magnetske rezonancije (MR) i serumska vrijednosti kemerina. U svim ispitanicima je otkazana kompletna krvna slika uz procjenu serumskih razina kemerina i fibrinogena. Učinjeni su regresijski modeli za procjenu serumskih razina kemerina uz kontrolu prema spolu, dobi i antropometrijskim mjerama. Serumski kemerin srednje vrijednosti iznosio je 141 (125-195) ng/mL. Serumski kemerin srednje vrijednosti srednje intenzitet je koreliran s volumenom abdominalnog visceralnog (VMT) i subkutanog (SMT) masnog tkiva. Dva najvažnijih prediktora serumskog kemerina bili su volumen SMT i opseg natkoljnice. Nezavisno od volumetrijskih mjerena, pušenje, slana hrana i preferiranje okusa jednostavne prehrane imali su utjecaj na serumski kemerin. Serumski kemerin je jači je koreliran s volumenom SMT nego VMT, dok je nezavisno od volumena masnog tkiva određene životne navike utječu na razine serumskog kemerina.

Ključne riječi: Pretilost; Citokini – krv; Magnetska rezonancija, snimanje; Fibrinogen; Abdominalno salo; Adipokini; Intra-abdominalno salo; Način života; Pušenje