Serum and adipose tissue chemerin is differentially related to insulin sensitivity

Monika Karczewska-Kupczewska¹, Agnieszka Nikołajuk², Magdalena Stefanowicz³, Natalia Matulewicz³, Irina Kowalska¹, Marek Strączkowski²

¹ Department of Internal Medicine and Metabolic Diseases, Medical University of Białystok, Poland;
² Department of Prophylaxis of Metabolic Diseases, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland;
³ Department of Metabolic Diseases, Medical University of Białystok, Poland.

Corresponding author:
Monika Karczewska-Kupczewska, MD, PhD
Department of Internal Medicine and Metabolic Diseases, Medical University of Białystok, Poland;
M.C. Skłodowskiej 24a, 15-276 Białystok, Poland
Phone: +48 85 8317761 Fax: +48 85 8317768 e-mail: monika3101@wp.pl

Short title: Serum and adipose tissue chemerin in obesity
Keywords: chemerin, insulin sensitivity, obesity, adipose tissue, adipogenesis

Word count: 3939; Figure 1; Table 5.
Abstract

Objective: The aim of the study was to assess serum chemerin concentration and subcutaneous adipose tissue (SAT) chemerin expression in relation to insulin sensitivity and obesity in young healthy subjects.

Design: We performed a cross-sectional study including 128 subjects, 44 normal-weight, 44 with overweight and 40 with obesity.

Methods: Hyperinsulinemic-euglycemic clamp and SAT biopsy were performed. Next, 30 subjects with obesity underwent 12-week weight-reducing dietary intervention.

Results: Serum chemerin was higher and SAT chemerin expression was lower in subjects with obesity in comparison with other groups. The relationships of serum chemerin with its SAT expression and insulin sensitivity were positive in individuals with normal-weight and with overweight and negative in individuals with obesity. In the entire study population, serum chemerin was also positively related to hsCRP, serum fetuin A and alanine aminotransferase. SAT chemerin was positively related to insulin sensitivity, SAT insulin signaling and adipogenic genes. Weight loss decreased serum chemerin, whereas SAT chemerin increased in subjects with the highest increase in insulin sensitivity.

Conclusions: Serum and SAT chemerin is differentially associated with insulin sensitivity and the relationship between serum chemerin and insulin sensitivity depends on adiposity. SAT chemerin is positively associated with insulin sensitivity across the wide range of BMI and may be proposed as a biomarker of metabolically healthy SAT. Our results suggest that SAT is not the main source of serum chemerin in obesity.
Introduction

Chemerin, also known as retinoic acid receptor responder protein 2 (RARRES2) or RAR-responsive protein TIG2, is a protein that in humans is encoded by the RARRES2 gene. It was initially identified as retinoid responsive gene present in psoriatic skin lesions (1). In 2007, chemerin was identified as an adipokine (2). It is also expressed at the high level in the liver (3). Furthermore, its expression in others tissues such as placenta, ovary has been confirmed (4, 5). Chemerin is initially secreted as an inactive form, prochemerin, and then processed to an active form via a cleavage of five to nine C-terminal amino acids by serine proteases such as mast cell tryptase and elastase (6). The primary function of chemerin involves chemoattraction of macrophages and dendritic cells during the immune response (7). Recent data indicate that chemerin is involved in the regulation of adipogenesis, adipocyte metabolism and glucose homeostasis (2).

Increased circulating chemerin concentration has been reported in obesity (8-10) and related comorbidities such as metabolic syndrome (11), nonalcoholic fatty liver disease (NAFLD) (12) and type 2 diabetes (9, 13). Systemic chemerin concentrations decreased after weight loss induced by bariatric surgery (12, 14). Furthermore, positive correlations between serum chemerin and BMI, blood triglyceride level, waist-to-hip ratio, blood pressure and an inverse correlation with HDL-cholesterol, were observed in different populations (8, 11, 13). Systemic chemerin levels were also found to be associated with markers of fatty liver disease (12).

The main pathophysiological factor of obesity-related metabolic complications is insulin resistance. As chemerin is related to the sequelae of insulin resistance, the association between chemerin and insulin sensitivity seems to be of importance. However, studies regarding this relationship are inconsistent. Significant positive correlation between systemic chemerin and insulin resistance has been reported (11, 12, 15, 16), however, not all studies
confirm this relationship (17, 18). Furthermore, data regarding the correlations between adipose tissue (AT) chemerin expression and insulin sensitivity are limited and inconclusive (4, 9, 18).

Although numerous investigators have shown increased blood chemerin concentration in humans with obesity (8-10), the data on AT chemerin expression are contradictory. Increased (9, 19) or decreased (15) chemerin mRNA expression has been reported in white AT in obesity. The main source of circulating chemerin in obesity is also not clear. Both positive (9, 18) and negative (17) correlations between serum chemerin and AT chemerin expression have been observed in humans.

The difference in results may arise from different inclusion in different studies. The data on chemerin come mainly from studies on humans with morbid obesity or with obesity-related comorbidities such as NAFLD, type 2 diabetes. The method of measurement of insulin sensitivity may also be of importance. Importantly, nobody has been studied serum chemerin level together with its AT expression in young people without overt metabolic disturbances.

It has not been also elucidated how chemerin influences insulin sensitivity. According to the experimental studies, the potential link between chemerin and insulin sensitivity may be adipogenesis. Chemerin or chemerin receptor knockdown impaired differentiation of 3T3-L1 cells and attenuated the expression of adipocyte gens involved in glucose and lipid homeostasis (2).

For the maintaining of proper insulin action, it is essential to maintain adipogenesis, i.e. the ability of AT to recruit new fat cells, as the disturbances in this process may lead to an abnormal enlargement of existing adipocytes and to an ectopic lipid accumulation and development of insulin resistance. Preadipocytes differentiation into mature fat cells is controlled by transcription factors, in particular, CCAAT/enhancer binding protein (C/EBP)-α, -β and peroxisome proliferator activator receptor (PPAR)-γ (20). An impaired adipogenesis
and AT dysfunction manifests with lower expression of transcription factors regulating adipogenesis and genes characteristic for mature adipocytes such as the genes encoding components of insulin signaling (21). The relationship between genes associated with adipogenesis and chemerin has not been studied in humans to date.

The above data indicate that the role of chemerin in obesity and insulin resistance is still unclear and remains to be elucidated. Therefore, to avoid confounding factors, the aim of the present study was to assess serum chemerin concentration and subcutaneous adipose tissue (SAT) chemerin expression in young subjects without overt metabolic disturbances, but with a different degree of insulin sensitivity. Furthermore, we aimed to analyze the association between SAT chemerin and adipogenesis in humans.

**Materials and methods**

**Study group**

The study group comprised 128 healthy young subjects (mean age, 26.45 ± 6.30 years), 44 normal-weight (body mass index (BMI) < 25 kg/m², 32 males and 12 females), 44 with overweight (30 males and 14 females) and 40 with obesity (23 males and 17 females). All study participants were nonsmokers, without serious diseases, morbid obesity, impaired glucose tolerance or diabetes, and were not taking any drugs. Body of the subjects had remained stable (± 1 kg) for at least 3 months prior to the study. Participants underwent clinical examination, anthropometric measurements and appropriate laboratory tests (22, 23). Subjects were excluded if they had any inflammatory disease within the last 3 months. All subjects had no clinical and laboratory signs of inflammation and had not taken anti-inflammatory drugs within the last 3 months. A standard oral glucose tolerance test (OGTT)
was performed and all subjects had normal glucose tolerance according to World Health Organization criteria. All the studies were performed after overnight fast.

Studies have been performed according to the Declaration of Helsinki. The study protocol was approved by the local ethics committee of the Medical University of Bialystok, Poland. A written informed consent was obtained from all individual participants included in the study.

*Dietary intervention program*

Twelve-week dietary intervention program consisted of individually planned low-calorie diet (20 kcal per kg of proper body weight), as described previously (23). All analyses described below were performed before and after dietary intervention in 30 subjects with obesity, which completed the program.

*Insulin sensitivity and SAT biopsy*

Insulin sensitivity measurement with the 2 h hyperinsulinemic-euglycemic clamp and SAT biopsy were performed as described (22). Insulin sensitivity (M value) was calculated per fat-free mass (ffm).

*Biochemical measurements*

Plasma glucose, serum insulin, lipids and high-sensitive C-reactive protein (hsCRP) were measured as described (23). Total serum chemerin was measured with ELISA kit (Biovendor, Brno, Czech Republic) with sensitivity of 0,1 ng/ml and with intra-assay and inter-assay coefficients of variation (CVs) below 7.0 % and 8.3 % respectively. Serum fetuin A was measured with ELISA kit (R&D systems, Minneapolis, MN, USA) with detection limit 0,62 ng/ml and with intra-assay and inter-assay CVs below 4.9 % and 8.4 % respectively.

*Isolation of RNA from SAT and determination of gene expression*
Total RNA was isolated from SAT as described (22). AT mRNA expression of the gene encoding chemerin (RARRES2), insulin signaling (IRS1, IRS2, PIK3CA, AKT2, SLC2A4) and adipogenic (CEBPA, CEBPB, PPARG) genes was analyzed with quantitative Real Time PCR. The samples were quantified with the Light Cycler 480 II Real-Time PCR Instrument (Roche Diagnostics, GmbH, Mannheim, Germany) using Roche LightCycler480 Probes Master (Roche Diagnostics GmbH). For the determination of SAT RARRES2 expression we used primers: forward 5'-AACTGGGCTCTGAGGACAAA, reverse 5’-CCGCAGAACTTGGGTCTC, UPL probe #43. SAT expression of insulin signaling and adipogenic genes was measured using gene specific primers and probes, which were reported previously (22). All samples were run in triplicate and average values were calculated. All results were normalized to the levels of PGK1, since its expression was the most stable among three housekeeping genes tested.

Statistical analysis

The statistics were performed with the STATISTICA 12.5 (Statsoft, Krakow, Poland). All data are presented as mean ± SD. The variables, which did not have normal distribution (including serum and SAT chemerin) were log-transformed before analyses. For the purpose of the data presentation, absolute values are shown in the Results. Differences between the groups were analyzed with one-way ANOVA, followed by post-hoc Tukey test. To adjust for the effect of age and sex, we used ANCOVA. Differences before and after weight loss program were assessed with the paired Student’s t-test. Relationships between variables were studied with the Pearson product moment correlation analysis and with multiple regression analysis with the adjustment for BMI (or for the change in BMI after weight loss). The level of significance was accepted at p value lower than 0.05.
Results

Characteristics of the study groups

Characteristics of the study groups is presented in Table 1. Insulin sensitivity was lower in the groups with overweight and with obesity in comparison with the normal-weight group (p=0.032 and p<0.0001, respectively) and in the group with obesity in comparison with the group with overweight (p=0.009). Serum fetuin A concentration was higher in the groups with overweight and with obesity in comparison with the normal-weight group (p=0.005 and p<0.0001, respectively) and in the group with obesity in comparison with the group with overweight (p=0.004) (Table 1). All the differences remained significant after adjustment for age and sex.

Serum chemerin concentration and SAT chemerin expression

Total serum chemerin was higher in individuals with obesity in comparison with individuals with normal-weight and with overweight (p<0.0001 and p=0.003, respectively, Figure 1A). In contrast, SAT chemerin expression was lower in subjects with obesity in comparison with subjects with normal-weight and with overweight (both p<0.0001, Figure 1B). All the differences remained significant after adjustment for age and sex.

SAT insulin signaling and adipogenic gene expression

There were differences among the groups in SAT expression of insulin signaling genes: IRS1, IRS2, PIK3CA, AKT2 and SLC2A4 expression was lower in the groups with overweight and with obesity in comparison with the normal-weight group (all p<0.05), whereas IRS2 and SLC2A4 expression was also lower in the group with obesity in comparison with the group with overweight (p<0.05) (Table 2).
Similarly to insulin signaling, there were also differences in SAT adipogenic gene expression: \textit{CEBPA}, \textit{CEBPB} and \textit{PPARG} expression was lower in the groups with overweight and with obesity in comparison with the normal-weight group (both p<0.05), and \textit{CEBPA} and \textit{PPARG} expression was also lower in the group with obesity in comparison with the group with overweight (all p<0.05) (Table 2).

The relationships between serum and SAT chemerin and other estimated parameters

Serum chemerin was not related to its AT expression in the entire study population (Table 3). However, in subgroup analysis the relationship between serum chemerin and its SAT expression was positive in the groups with normal-weight and with overweight and negative in the group with obesity (Table 3).

In the entire study population, serum chemerin was not related to insulin sensitivity (Table 3). However, the relationship between serum chemerin and insulin sensitivity was positive in the groups with normal-weight and with overweight and negative in the group with obesity (Table 3).

Serum chemerin was positively related to BMI, waist circumference, percent of body fat, triglycerides, serum hsCRP, serum fetuin A concentration and alanine aminotransferase (AlAt) activity in the entire study group (Table 3). The relationships of serum chemerin with hsCRP (\(\beta=0.18, p=0.02\)), fetuin A (\(\beta=0.25, p=0.004\)) and AlAt (\(\beta=0.21, p=0.01\)) were independent of BMI.

In contrast, SAT chemerin expression was positively related to insulin sensitivity in the entire study group and in the subgroups with normal-weight, with overweight and with obesity (Table 4). SAT chemerin was also negatively related to BMI, waist circumference, percent of body fat (Table 4). There were significant positive correlations between SAT expression of chemerin and insulin signaling and adipogenic genes (Table 4).
regression analysis, the relationships of SAT chemerin with insulin sensitivity (β=0.39, p<0.0001) as well as with SAT *IRSI* (β=0.35, p=0.0001), *IRS2* (β=0.17, p=0.045), *PIK3CA* (β=0.24, p=0.013), *CEBPA* (β=0.33, p=0.0002), *CEBPB* (β=0.20, p=0.033) and *PPARG* (β=0.36, p=0.0001) were independent of BMI.

*The effect of dietary intervention on serum and SAT chemerin*

In 30 subjects with obesity, dietary intervention program resulted in a reduction in body weight by 11.4% (101 ± 15.2 kg vs. 89.6 ± 13.5 kg, p<0.0001) and an increase in insulin sensitivity by 30.2% (Table 5). It was accompanied by the reduction in serum chemerin by 10.2% (Table 5). The change in serum chemerin was positively related to the concurrent change in BMI (r=0.45, p=0.013) and negatively with insulin sensitivity (r=-0.44, p=0.017), overall. An inverse association with the change in insulin sensitivity mean that the higher increase in M value, the higher decrease in serum chemerin. The association of the change in insulin sensitivity with the change in serum chemerin was independent of the concurrent change in BMI (β=-0.39, p=0.021).

SAT chemerin expression did not change in response to weight loss when the entire group with obesity was analyzed (Table 5). However, in subjects in the highest tertile of the change in insulin sensitivity (i.e. $\Delta M_{\text{ffm}} > +3.298$ mg/kg ffm/min) there was an increase in SAT chemerin expression (0.72 ± 0.29 vs. 0.97 ± 0.32 A.U., p=0.023) after weight loss.

**Discussion**

The main finding of our study is that serum and SAT chemerin is differentially associated with insulin sensitivity and that SAT expression of chemerin is positively correlated with adipogenic and insulin signaling genes independently of BMI in humans.
It should be noted that the results of simultaneous measurements of circulating and SAT chemerin in young population without confounding factors such as metabolic disturbances have not been published so far. In agreement with our results, higher circulating chemerin levels in humans with obesity than in controls without obesity were also observed by other researchers (8-10). Positive correlations between serum chemerin and parameters of obesity in populations of subjects with different fat mass and varying BMI were also reported in other studies (8-12). Although studies on circulating chemerin in obesity are quite consistent, the data regarding the association between SAT chemerin and fat mass are conflicting.

Similarly to our data, decreased chemerin expression in SAT (15) was observed in pregnant women with obesity and with normal glucose tolerance compared with normal-weight and normal glucose tolerant pregnant women. In this study, no correlation was found between chemerin mRNA/protein expression in SAT and BMI. On the other hand, Chakaroun et al. observed increased SAT chemerin expression and its positive correlation with BMI in population with the wide range of BMI (9). However, these authors examined subjects older than in our study and the group with obesity in their study included subjects with morbid obesity, whereas in our study morbid obesity was an exclusion criterion. Such differences in subjects’ characteristics may likely influence the results. Chemerin regulates adipogenesis, so it is also possible that the differences in results may be associated with different types of adipose tissue expansion, i.e. hypertrophy or hyperplasia. Furthermore, chemerin is induced by proinflammatory cytokines, so the degree of inflammation in adipose tissue may influence the results.

We discovered that serum chemerin was not related to insulin sensitivity, in the entire study population, which was due to the opposite correlations between serum chemerin and insulin sensitivity in the subgroups of normal-weight and overweight and in the subgroup of
obese subjects. In contrast, SAT chemerin expression was positively related to insulin sensitivity in the entire study group and in the subgroups. It has been found that circulating chemerin positively correlated with HOMA-IR (4,11,12,15), and negatively with insulin sensitivity measured by the clamp (16). However, no relationships between circulating chemerin and insulin sensitivity indices have been also observed (17,18). The positive relationship between SAT chemerin and insulin resistance has been described (18), but the lack of such correlation was also observed (4).

Based on our investigations, it appears that chemerin, acting locally in SAT exerts positive effect on whole-body insulin sensitivity, although the exact causality cannot be established. It could be due to positive influence on adipogenesis which results proper AT function. In agreement with our suspicion, there are experimental studies which indicate that chemerin expression and secretion increase dramatically with adipogenesis and loss of chemerin expression in preadipocytes disrupts their differentiation into mature adipocytes (2). Takahashi et al. also discovered that chemerin enhanced insulin signaling and potentiated insulin-stimulated glucose uptake in 3T3-L1 adipocytes (24). We for the first time have found that SAT expression of chemerin was positively correlated with adipogenic and insulin signaling genes in humans. This independence of the degree of adiposity may suggest that SAT chemerin is a positive marker of insulin sensitivity and the decreased SAT chemerin mRNA level in obese humans may be associated with impaired adipogenesis. In fact, a decrease in the expression of genes involved in adipogenesis was observed in SAT in our group of subjects with obesity. However, similar changes regarding these markers of proper adipogenesis were observed in SAT of the group with overweight, despite unchanged expression of chemerin. The degree of impairment of adipogenesis could therefore be of importance. SAT chemerin could be an initial marker of SAT dysfunction which results in metabolic complications. Indeed, our subjects with obesity had the lowest insulin sensitivity
and expression of genes associated with adipocyte differentiation and maturation in comparison with others study groups. Thus, we propose SAT chemerin as a biomarker of metabolically healthy SAT because it may reflect the preserved insulin action and adipogenesis in SAT. The fact that the lower SAT expression of chemerin was found in women with gestational diabetes mellitus (GDM) in comparison with normal glucose tolerant women in pregnancy is in agreement with this hypothesis (15). Interestingly, we also found an increase in SAT chemerin expression in subjects in the highest increase in insulin sensitivity after weight loss. Our study does not reveal the mechanism of SAT chemerin increase in response to weight loss. However, we hypothesize that more profound metabolic changes are necessary to induce an increase in SAT chemerin. It is also possible that an increase in SAT chemerin induces further improvement in insulin sensitivity. Nevertheless, increase in SAT chemerin in subjects with the highest tertile of the change in insulin sensitivity after weight loss further supports our hypothesis that SAT chemerin is a biomarker of healthy AT.

Elevated systemic chemerin and its negative correlation with insulin sensitivity in the group with obesity seem to reflect the degree of metabolic complications associated with lipotoxicity and ectopic fat depositions due to AT dysfunction. It has been discovered that serum chemerin was significantly lower in metabolically healthy subjects with morbid obesity in comparison with metabolically unhealthy group with morbid obesity and with metabolic syndrome (26). Furthermore, elevated serum chemerin levels in metabolic syndrome and type 2 diabetes as compared with controls without metabolic disturbances have been observed (11,15). Additionally, circulating chemerin increases parallel to worsening of glucose tolerance status (27). Indeed, we observed the reduction in serum chemerin after weight loss with the concurrent improvement in whole-body insulin sensitivity and the decrease in serum chemerin was related to the increase in insulin sensitivity independently of the concurrent change in BMI. Decrease in serum chemerin concentration after weight loss may be
interpreted as a step to normalization of metabolic disturbances associated with an increased body weight. Similar findings were also observed by other authors (9,12,14).

It has been reported that chemerin inhibits glucose uptake and induces insulin resistance in skeletal muscle (28), which suggests that the metabolic effects of chemerin may be tissue-dependent. Chemoattractive actions of chemerin could also be of importance regarding insulin resistance. We found that serum chemerin was positively associated with CRP. It is also possible that the degree of systemic chemerin elevation may determine, which chemerin effects prevail and how this protein influences whole-body insulin sensitivity. Furthermore, differentially cleaved chemerin forms are present in the circulation, with different biological activities (29). These findings may potentially explain different direction of the correlation between serum chemerin and insulin sensitivity in the groups of normal-weight and overweight subjects and in the group of obese subjects. These issues remain relatively unexplored in humans and should be studied further.

We found low chemerin expression in SAT and high serum chemerin concentrations only in subjects with obesity. The inverse correlation between circulating chemerin and subcutaneous SAT chemerin expression in humans with wide range of obesity was found also by other investigators (17). However, in this study the differences in serum and SAT expression between controls and subjects with obesity were not presented. In our study we observed that weight loss resulted in a decrease in serum chemerin concentration, whereas SAT chemerin expression did not change and even increased in the subgroup with the highest improvement in insulin sensitivity.

The above data suggest that SAT is not the main source of circulating chemerin in obesity. Given the fact that chemerin is also highly expressed in the liver (3), both in fetal and adult human tissues (30), it is possible that the liver might significantly contribute to systemic
levels of chemerin. It has been also shown that chemerin levels were similar in portal vein blood and systemic venous blood and significantly elevated in hepatic vein (31). Insufficiency of SAT results in an ectopic accumulation of fat and in consequence leads to lipotoxicity and development of insulin resistance. Liver affected by fat accumulation and insulin resistance could be responsible for increase in systemic chemerin. Chemerin mRNA induced in the liver of rodent fed a high fat diet (32). Recent studies have been reported elevated serum or hepatic expression levels of chemerin in NAFLD and found positive correlations with NAFLD active score or hepatic inflammation (12,33,34). We observed positive correlations of serum chemerin with AlAt and fetuin A, which are predictors of liver fat (35,36), as well as with circulating CRP, which is secreted mainly by the liver. Interestingly, Bekaert et al. observed an inverse relationship between visceral adipose tissue (VAT) chemerin expression and NAFLD activity score. In this study, VAT chemerin explained, at least partly, the relationship between NAFLD and insulin resistance (37).

However, one cannot exclude VAT as a source of increased serum chemerin concentration in obesity as it was also demonstrated that circulating chemerin was positively related to its expression in omental adipose tissue, but not in SAT, in subjects with obesity (9). Both decreased (4) and increased (17) chemerin expression in SAT in comparison with its expression in VAT has been observed. No fat-depot-specific differences in chemerin mRNA levels were also detected (18). In the study of Svennson et al. chemerin secretion from VAT was marginally higher than from subcutaneous depot over 24 h, however, the pattern of chemerin secretion was very similar in both depots (25). Tsiotra et al. (4) observed that VAT chemerin, but not SAT chemerin, was higher in obese women with GDM in comparison with non-obese pregnant women with normal glucose tolerance. However, chemerin expression both in VAT and SAT was not significantly different in obese women with GDM in
comparison with the group of obese women with normal glucose tolerance as well as in the non-obese group with GDM.

Our study has several limitations. We did not measure VAT chemerin expression. We also did not measure SAT chemerin protein expression, which was impossible due to the limited tissue availability. Due to the cross-sectional design of our study, one may not establish the causality between chemerin and insulin sensitivity.

We conclude that serum and SAT chemerin is differentially associated with insulin sensitivity. SAT chemerin is positively associated with insulin sensitivity and markers of adipogenesis across the wide range of BMI and may be proposed as a biomarker of metabolically healthy SAT. The relationship between serum chemerin and insulin sensitivity depends on adiposity. Serum chemerin may be an early marker of SAT insufficiency and whole-body metabolic disturbances. Our results also suggest that SAT is not the main source of serum chemerin in obesity.
Declaration of interest:

The authors declare that there is no conflict of interest.

Funding:

Supported by The Grant UDA-POIG.01.03.01-00-128/08; from the Program Innovative Economy 2007-2013; part-financed by the European Union within the European Regional Development Fund; by the Grant NCN number 2011/03/B/NZ7/04980 from the National Science Centre, Poland and by the statutory funds of the Medical University of Bialystok, Poland (N/ST/ZB/17/001/1173 and N/ST/ZB/17/002/1173).
References

1. Nagpal S, Patel S, Jacobe H, DiSepio D, Ghosn C, Malhotra M, Teng M, Duvic M & Chandraratna RA. Tazarotene-induced gene 2 (TIG2), a novel retinoid-responsive gene in skin. *Journal of Investigative Dermatology* 1997 109 91-95.

2. Goralski KB, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, Parlee SD, Muruganandan S & Sinal CJ. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. *Journal of Biological Chemistry* 2007 282 28175-28188.

3. Krautbauer S, Wanninger J, Eisinger K, Hader Y, Beck M, Kopp A, Schmid A, Weiss TS, Dorn C & Buechler C. Chemerin is highly expressed in hepatocytes and is induced in non-alcoholic steatohepatitis liver. *Experimental and Molecular Pathology* 2013 95 199-205.

4. Tsiotra PC, Halvatsiotis P, Patsouras K, Maratou E, Salamalekis G, Raptis SA, Dimitriadis G & Boutati E. Circulating adipokines and mRNA expression in adipose tissue and the placenta in women with gestational diabetes mellitus. *Peptides* 2018 101 157-166.

5. Reverchon M, Cornuau M, Ramé C, Guerif F, Royère D & Dupont J. Chemerin inhibits IGF-1-induced progesterone and estradiol secretion in human granulosa cells. *Human Reproduction* 2012 27 1790-1800.

6. Zabel BA, Allen SJ, Kulig P, Allen JA, Cichy J, Handel TM & Butcher EC. Chemerin activation by serine proteases of the coagulation, fibrinolytic, and inflammatory cascades. *Journal of Biological Chemistry* 2005 280 34661-34666.

7. Wittamer V, Franssen JD, Vulcano M, Mirjolet JF, Le Poul E, Migeotte I, Brézillon S, Tyldesley R, Blanpain C, Detheux M, et al. Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. *Journal of Experimental Medicine* 2003 198 977-985.
8. Bozaoglu K, Segal D, Shields KA, Cummings N, Curran JE, Comuzzie AG, Mahaney MC, Rainwater DL, VandeBerg JL, MacCluer JW, et al. Chemerin is associated with metabolic syndrome phenotypes in a Mexican-American population. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 3085-3088.

9. Chakaroun R, Raschpichler M, Klöting N, Oberbach A, Flehmig G, Kern M, Schön MR, Shang E, Lohmann T, Dreßler M, et al. Effects of weight loss and exercise on chemerin serum concentrations and adipose tissue expression in human obesity. *Metabolism* 2012 **61** 706-714.

10. Sledzinski T, Korczynska J, Hallmann A, Kaska L, Proczko-Markuszewska M, Stefaniak T, Sledzinski M & Swierczynski J. The increase of serum chemerin concentration is mainly associated with the increase of body mass index in obese, non-diabetic subjects. *Journal of Endocrinological Investigation* 2013 **36** 428-434.

11. Jialal I, Devaraj S, Kaur H, Adams-Huet B & Bremer AA. Increased chemerin and decreased omentin-1 in both adipose tissue and plasma in nascent metabolic syndrome. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** E514-E517.

12. Sell H, Divoux A, Poitou C, Basdevant A, Bouillot JL, Bedossa P, Tordjman J, Eckel J & Clément K. Chemerin correlates with markers for fatty liver in morbidly obese patients and strongly decreases after weight loss induced by bariatric surgery. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 2892-2896.

13. Bozaoglu K, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, Walder K & Segal D. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. *Endocrinology* 2007 **148** 4687-4694.

14. Ress C, Tschoner A, Engl J, Klaus A, Tilg H, Ebenbichler CF, Patsch JR & Kaser S. Effect of bariatric surgery on circulating chemerin levels. *European Journal of Clinical Investigation* 2010 **40** 277-280.
15. Li XM, Ji H, Li CJ, Wang PH, Yu P & Yu DM. Chemerin expression in Chinese pregnant women with and without gestational diabetes mellitus. *Annales d'Endocrinologie* 2015 **76** 19-24.

16. Ouwens DM, Bekaert M, Lapauw B, Van Nieuwenhove Y, Lehr S, Hartwig S, Calders P, Kaufman JM, Sell H, Eckel J, et al. Chemerin as biomarker for insulin sensitivity in males without typical characteristics of metabolic syndrome. *Archives of Physiology and Biochemistry* 2012 **118** 135-138.

17. Alfadda AA, Sallam RM, Chishti MA, Moustafa AS, Fatma S, Alomaim WS, Al-Naami MY, Bassas AF, Chrousos GP & Jo H. Differential patterns of serum concentration and adipose tissue expression of chemerin in obesity: adipose depot specificity and gender dimorphism. *Molecules and Cells* 2012 **33** 591-596.

18. Tan BK, Chen J, Farhatullah S, Adya R, Kaur J, Heutling D, Lewandowski KC, O'Hare JP, Lehnert H & Randeva HS. Insulin and metformin regulate circulating and adipose tissue chemerin. *Diabetes* 2009 **58** 1971-1977.

19. Bremer AA & Jialal I. Adipose tissue dysfunction in nascent metabolic syndrome. *Journal of Obesity* 2013 **2013** 393192.

20. Lefterova MI, Zhang Y, Steger DJ, Schupp M, Schug J, Cristancho A, Feng D, Zhuo D, Stoeckert CJ Jr, Liu XS, et al. PPARgamma and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. *Genes & Development* 2008 **22** 2941-2952.

21. Dubois SG, Heilbronn LK, Smith SR, Albu JB, Kelley DE, Ravussin E & Look AHEAD Adipose Research Group. Decreased expression of adipogenic genes in obese subjects with type 2 diabetes. *Obesity (Silver Spring)* 2006 **14** 1543-1552.
22. Matulewicz N, Stefanowicz M, Nikołajuk A & Karczewska-Kupczewska M. Markers of adipogenesis, but not inflammation in adipose tissue, are independently related to insulin sensitivity. *Journal of Clinical Endocrinology and Metabolism* 2017 **102** 3040-3049.

23. Strączkowski M, Nikołajuk A, Majewski R, Filarski R, Stefanowicz M, Matulewicz N & Karczewska-Kupczewska M. The effect of weight loss, with or without β-glucan addition, on adipose tissue inflammatory gene expression. *Endocrine* 2018 **61** 275-284.

24. Takahashi M, Takahashi Y, Takahashi K, Zolotaryov FN, Hong KS, Kitazawa R, Iida K, Okimura Y, Kaji H, Kitazawa S, et al. Chemerin enhances insulin signaling and potentiates insulin-stimulated glucose uptake in 3T3-L1 adipocytes. *FEBS Letters* 2008 **582** 573-578.

25. Svensson H, Odén B, Edén S & Lönn M. Adiponectin, chemerin, cytokines, and dipeptidyl peptidase 4 are released from human adipose tissue in a depot-dependent manner: an in vitro system including human serum albumin. *BMC Endocrine Disorders* 2014 **14** 7.

26. Cătoi AF, Pârvu AE, Andreicuț AD, Mironiuc A, Crăciun A, Cătoi C & Pop ID. Metabolically Healthy versus Unhealthy Morbidly Obese: Chronic Inflammation, Nitro-Oxidative Stress, and Insulin Resistance. *Nutrients* 2018 **10** E1199.

27. Tönjes A, Fasshauer M, Kratzsch J, Stumvoll M & Blüher M. Adipokine pattern in subjects with impaired fasting glucose and impaired glucose tolerance in comparison to normal glucose tolerance and diabetes. *PLoS One* 2010 **5** e13911.

28. Sell H, Laurencikiene J, Taube A, Eckardt K, Cramer A, Horrighs A, Arner P & Eckel J. Chemerin is a novel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. *Diabetes* 2009 **58** 2731-2740.

29. Chang SS, Eisenberg D, Zhao L, Adams C, Leib R, Morser J & Leung L. Chemerin activation in human obesity. *Obesity (Silver Spring)* 2016 **24** 1522-1529.
30. Kasher-Meron M, Mazaki-Tovi S, Barhod E, Hemi R, Haas J, Gat I, Zilberberg E, Yinon Y, Karasik A & Kanety H. Chemerin concentrations in maternal and fetal compartments: implications for metabolic adaptations to normal human pregnancy. J Perinat Med 2014 42 371-378.

31. Weigert J, Neumeier M, Wanninger J, Filarsky M, Bauer S, Wiest R, Farkas S, Scherer MN, Schäffler A, Aslanidis C, et al. Systemic chemerin is related to inflammation rather than obesity in type 2 diabetes. Clinical Endocrinology 2010 72 342-348.

32. Ernst MC, Issa M, Goralski KB & Sinal CJ. Chemerin exacerbates glucose intolerance in mouse models of obesity and diabetes. Endocrinology 2010 151 1998-2007.

33. Kukla M, Zwirska-Korczała K, Hartleb M, Waluga M, Chwist A, Kajor M, Ciupinska-Kajor M, Berdowska A, Wozniak-Grygiel E & Buldak R. Serum chemerin and vaspin in non-alcoholic fatty liver disease. Scandinavian Journal of Gastroenterology 2010 45 235-242.

34. Döcke S, Lock JF, Birkenfeld AL, Hoppe S, Lieske S, Rieger A, Raschzok N, Sauer IM, Florian S, Osterhoff MA, et al. Elevated hepatic chemerin mRNA expression in human non-alcoholic fatty liver disease. European Journal of Endocrinology 2013 169 547-557.

35. Maximos M, Bril F, Portillo Sanchez P, Lomonaco R, Orsak B, Biernacki D, Suman A, Weber M & Cusi K. The role of liver fat and insulin resistance as determinants of plasma aminotransferase elevation in nonalcoholic fatty liver disease. Hepatology 2015 61 153-160.

36. von Loeffelholz C, Horn P, Birkenfeld AL, Claus RA, Metzing BU, Döcke S, Jahreis G, Heller R, Hoppe S, Stockmann M, et al. Fetuin A is a Predictor of Liver Fat in Preoperative Patients with Nonalcoholic Fatty Liver Disease. Journal of Investigative Surgery 2016 29 266-274.
37. Bekaert M, Ouwens DM, Hörbelt T, Van de Velde F, Fahlbusch P, Herzfeld de Wiza D, Van Nieuwenhove Y, Calders P, Praet M, Hoorens A, et al. Reduced expression of chemerin in visceral adipose tissue associates with hepatic steatosis in patients with obesity. *Obesity* 2016 **24** 2544-2552.
Figure legend

Figure 1. Serum chemerin concentration (A) and SAT RARRES2 expression (B) in subjects with normal-weight (n=44), with overweight (n=44) and with obesity (n=40).

* p<0.05 vs. the normal-weight group

# p<0.05 vs. the group with overweight
Table 1. Clinical and biochemical characteristics of the study groups.

|                           | Normal-weight (n=44) | Overweight (n=44) | Obesity (n=40) |
|---------------------------|----------------------|------------------|---------------|
| Age (years)               | 23.3±2.34            | 24.6±4.0         | 31.9±7.7*#    |
| BMI (kg/m\(^2\))         | 22.5±1.5             | 27.5±1.5*        | 33.9±2.5*#    |
| Waist circumference (cm)  | 81.8±4.7             | 94.3±7.6*        | 109±9.1*#     |
| % body fat                | 18.2±6.9             | 28.4±6.9*        | 38.2±7.2*#    |
| Fasting plasma glucose (mg/dL) | 85.8±7.2         | 88.2±9.7         | 92.6±8.1*#    |
| Plasma glucose at 120 min OGTT (mg/dL) | 79.3±16.6      | 85.5±16.3        | 92.7±18.1*    |
| Fasting serum insulin (µIU/mL) | 9.3±5.1           | 13.2±7.4*        | 15.3±4.4*     |
| M (mg/kg ffm/min)         | 8.77±2.79            | 7.29±2.93*       | 5.51±2.55*#   |
| Cholesterol (mg/dL)       | 167±30.9             | 174±27.4         | 192±26.1*#    |
| Triglycerides (mg/dL)     | 74.8±30.3            | 88.3±44.5        | 119±59.0*#    |
| HDL-cholesterol (mg/dL)   | 64.9±13.3            | 56.5±12.7*       | 51.5±9.5*     |
| LDL-cholesterol (mg/dL)   | 96.9±28.4            | 105±26.4         | 125±31.7*#    |
| hsCRP (mg/L)              | 0.43±0.28            | 0.65±0.48*       | 1.40±0.80*#   |
| Serum fetuin A (µg/mL)    | 674±171              | 822±266*         | 976±212*#     |
| AIAt (U/L)                | 187±7.77             | 25.4±11.8*       | 34.4±9.3*#    |

* p<0.05 vs. the normal-weight group  # p<0.05 vs. the group with overweight

BMI, body mass index; M, insulin sensitivity; ffm, fat-free mass; hsCRP, high-sensitive C-reactive protein; AIAt, alanine aminotransferase
Table 2. Adipose tissue insulin signaling and adipogenic gene expression in the studied groups (A.U.).

| Gene     | Normal-weight (n=44) | Overweight (n=44) | Obesity (n=40) |
|----------|----------------------|-------------------|---------------|
| IRS1     | 1.05±0.35            | 0.86±0.31*        | 0.72±0.27*    |
| IRS2     | 1.55±0.52            | 1.30±0.48*        | 0.94±0.30*#   |
| PIK3CA   | 1.23±0.36            | 1.08±0.31*        | 0.97±0.19*    |
| AKT2     | 1.12±0.48            | 0.87±0.37*        | 0.81±0.33*    |
| SLC2A4   | 2.74±1.18            | 1.62±1.20*        | 0.95±0.49*#   |
| CEBPA    | 1.20±0.30            | 0.96±0.26*        | 0.77±0.32*#   |
| CEBPB    | 1.09±0.50            | 0.78±0.44*        | 0.58±0.34*    |
| PPARG    | 1.22±0.36            | 1.04±0.36*        | 0.83±0.22*#   |

* p<0.05 vs. the normal-weight group
# p<0.05 vs. the group with overweight

A.U., arbitrary units
Table 3. Correlations between serum chemerin and other estimated parameters.

|                     | Entire study (n=128) | Normal-weight (n=44) | Overweight (n=44) | Obese (n=40) |
|---------------------|----------------------|----------------------|-------------------|--------------|
| Serum chemerin      |                      |                      |                   |              |
| BMI                 | **0.42**             | -0.16                | -0.03             | 0.31         |
| Waist circumference | **0.37**             | -0.11                | -0.02             | 0.13         |
| % body fat          | **0.38**             | 0.27                 | 0.01              | 0.04         |
| M                   | -0.08                | **0.31**             | **0.47**          | **-0.48**    |
| Triglycerides       | **0.20**             | 0.14                 | 0.09              | -0.03        |
| hsCRP               | **0.40**             | 0.18                 | 0.02              | **0.41**     |
| Fetuin A            | **0.39**             | 0.06                 | 0.19              | **0.45**     |
| AlAt                | **0.39**             | 0.07                 | 0.12              | **0.54**     |
| SAT RARRES2         | -0.09                | **0.57**             | **0.51**          | **-0.46**    |

Correlation coefficients (Pearson’s r) are shown in the Table. Significant correlations (p<0.05) are shown in bold.

BMI, body mass index; M, insulin sensitivity; hsCRP, high-sensitive C-reactive protein; AlAt, alanine aminotransferase; SAT, subcutaneous adipose tissue
Table 4. Correlations between SAT RARRES2 and other estimated parameters.

|                      | Entire study (n=128) | Normal-weight (n=44) | Overweight (n=44) | Obese (n=40) |
|----------------------|----------------------|----------------------|-------------------|--------------|
| BMI                  | -0.49                | -0.27                | -0.04             | -0.24        |
| Waist circumference  | -0.50                | -0.23                | -0.27             | -0.21        |
| % body fat           | -0.30                | 0.15                 | -0.09             | 0.12         |
| M                    | 0.51                 | 0.34                 | 0.57              | 0.33         |
| IRS1                 | 0.47                 | 0.41                 | 0.35              | 0.36         |
| IRS2                 | 0.40                 | 0.24                 | 0.21              | 0.26         |
| PIK3CA               | 0.36                 | 0.22                 | 0.27              | 0.38         |
| AKT2                 | 0.18                 | 0.18                 | -0.14             | 0.15         |
| SLC2A4               | 0.28                 | 0.18                 | -0.03             | 0.07         |
| CEBPA                | 0.50                 | 0.16                 | 0.23              | 0.62         |
| CEBPB                | 0.37                 | 0.19                 | 0.19              | 0.35         |
| PPARG                | 0.49                 | 0.32                 | 0.38              | 0.47         |

Correlation coefficients (Pearson’s r) are shown in the Table. Significant correlations (p<0.05) are shown in bold.

BMI, body mass index; M, insulin sensitivity
Table 5. The effect of 12-week dietary intervention on clinical and biochemical parameters, serum chemerin and SAT *RARRES2* in the obese group (n=30).

| Parameter                          | Before (n=30)  | After (n=30)  |
|-----------------------------------|----------------|---------------|
| BMI (kg/m²)                       | 34.4±2.32      | 30.3±2.30*    |
| Waist circumference (cm)          | 109±8.9        | 99.9±8.4*     |
| % body fat                        | 41.2±4.3       | 36.3±5.4*     |
| Fasting plasma glucose (mg/dL)    | 88.7±4.1       | 86.6±6.3      |
| Fasting serum insulin (μU/mL)     | 14.9±4.1       | 11.4±3.0*     |
| M (mg/kg ffm/min)                 | 6.06±2.71      | 7.89±2.71*    |
| Cholesterol (mg/dL)               | 194±26.5       | 175±31.2*     |
| Triglycerides (mg/dL)             | 114±58.9       | 93.1±58.2     |
| HDL-cholesterol (mg/dL)           | 51.8±9.2       | 48.1±9.3*     |
| LDL-cholesterol (mg/dL)           | 126±31.3       | 112±30.8      |
| hsCRP (mg/L)                      | 1.40±0.85      | 0.98±0.90*    |
| AIAt (U/L)                        | 34.2±8.9       | 19.0±8.2*     |
| Serum chemerin (ng/mL)            | 228±37.0       | 211±36.2*     |
| SAT *RARRES2* (A.U.)              | 0.87±0.37      | 0.91±0.34     |

* p<0.05 for the difference after vs. before 12-week dietary intervention (n=30)

BMI, body mass index; M, insulin sensitivity; ffm, fat-free mass; hsCRP, high-sensitive C-reactive protein; AIAt, alanine aminotransferase
