RESEARCH

Serum and adipose tissue chemerin is differentially related to insulin sensitivity

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Abstract

Objective: The aim of the study was to assess serum chemerin concentration and s.c. 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 with 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 relationship of serum chemerin with SAT expression and insulin sensitivity were positive in normal weight and overweight individuals, 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 a wide range of BMIs 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.

Key Words
- chemerin
- insulin sensitivity
- obesity
- adipose tissue
- adipogenesis

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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 a high level in the liver (3). Furthermore, its expression in other tissues such as placenta and ovary has been confirmed (4, 5). Chemerin is initially secreted as an inactive form, prochemerin, and is then processed to an active form via the 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 the 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, 9, 10) and related comorbidities such as metabolic syndrome (11), nonalcoholic fatty

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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 and 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, 9, 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 criteria in different studies. The data on chemerin come mainly from studies on humans with morbid obesity or with obesity-related comorbidities such as NAFLD and type 2 diabetes. The method of measurement of insulin sensitivity may also be of importance. Importantly, nobody has studied serum chemerin level together with its AT expression in young people without overt metabolic disturbances.

It has also not been elucidated how chemerin influences insulin sensitivity. According to 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 genes involved in glucose and lipid homeostasis (2).

For the maintenance of proper insulin action, it is essential to maintain adipogenesis (the ability of AT to recruit new fat cells), as disturbances in this process may lead to an abnormal enlargement of existing adipocytes, to an ectopic lipid accumulation and to the development of insulin resistance. Preadipocyte 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). Impaired adipogenesis and AT dysfunction manifest with lower expression of the transcription factors which regulate adipogenesis and the genes characteristic of mature adipocytes, such as the genes encoding components of the insulin signaling pathway (21). The relationship between genes associated with adipogenesis and chemerin has not been studied in humans to date.

The previously mentioned 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 s.c. 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 of 128 healthy young subjects (mean age, 26.45 ± 6.30 years), 44 with normal weight (BMI <25 kg/m^2, 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. The body weight 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 an overnight fast.

All 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

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Bialystok, Poland. Written informed consent was obtained from all individual participants included in the study.

Dietary intervention program

The 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 subsequently 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 previously (22). Insulin sensitivity (M value) was calculated per fat-free mass (ffm).

Biochemical measurements

Plasma glucose, serum insulin, lipids and high-sensitivity C-reactive protein (hsCRP) were measured as described previously (23). Total serum chemerin was measured with an ELISA kit (Biovendor, Brno, Czech Republic) with a 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 an ELISA kit (R&D systems) with a detection limit of 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 previously (22). AT mRNA expression of the gene encoding chemerin (*RARRES2*), insulin signaling (*IRS1*, *IRS2*, *PIK3CA*, *AKT2* and *SLC2A4*) and adipogenic (*CEBPA*, *CEBPB* and *PPARG*) genes were analyzed with quantitative real time PCR. The samples were quantified with a Light Cycler 480 II Real-Time PCR Instrument (Roche Diagnostics) using Roche LightCycler480 Probes Master (Roche Diagnostics). For the determination of SAT *RARRES2* expression we used the following 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 the three housekeeping genes tested.

Statistical analysis

The statistical analysis was performed with STATISTICA 12.5 (Statsoft, Krakow, Poland). All data are presented as mean ± S.D. The variables which did not have a normal distribution (including serum and SAT chemerin), were log-transformed before analyses. For the purpose of data presentation, absolute values are shown in the Results section. 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 the 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* values lower than 0.05.

Results

Characteristics of the study groups

The characteristics of the study groups are 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, Fig. 1A). In contrast, SAT chemerin expression was lower in subjects with obesity in comparison with

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

[a]P < 0.05 vs the normal-weight group; [b]P < 0.05 vs the group with overweight.
AlAt, alanine aminotransferase; ffm, fat-free mass; hsCRP, high-sensitive C-reactive protein; M, insulin sensitivity.

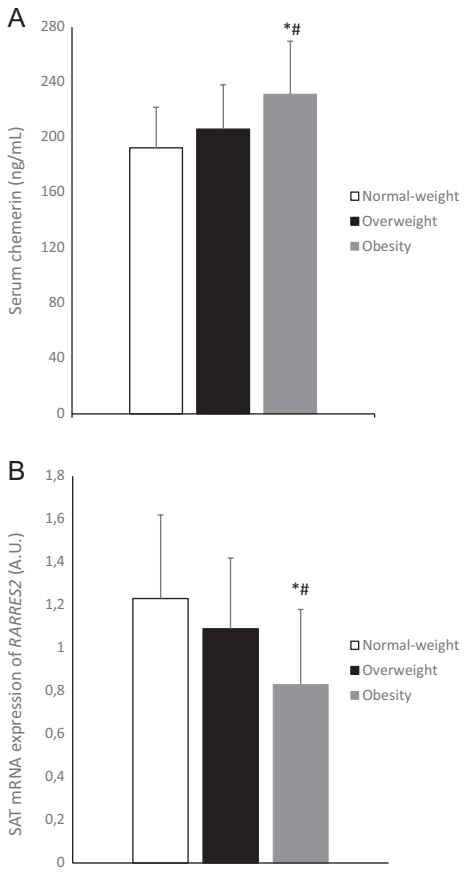

Figure 1
Serum chemerin concentration (A) and SAT *RARRES2* expression (B) in subjects with normal weight (n = 44), with overweight (n = 44) and with obesity (n = 40). *P < 0.05 vs the normal-weight group. #P < 0.05 vs the group with overweight.

subjects with normal weight and with overweight (both P<0.0001, Fig. 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 the 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: *CEBPA*, *CEBPB* and *PPARG* expression was lower in the groups with overweight and with obesity in comparison with the normal-weight group (both P<0.05), and *CEBPA* and *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

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Table 2 Adipose tissue insulin signaling and adipogenic gene expression in the studied groups (A.U.).

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

[a]$P < 0.05$ vs the normal-weight group; [b]$P < 0.05$ vs the group with overweight.
A.U., arbitrary units.

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, percentage body fat, triglycerides, serum hsCRP, serum fetuin A concentration and alanine aminotransferase (AlAt) activity in the entire study group (Table 3). The relationship of serum chemerin with hsCRP (β=0.18, P=0.02), fetuin A (β=0.25, P=0.004) and AlAt (β=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 and percentage body fat (Table 4). There were significant positive correlations between SAT expression of chemerin and insulin signaling and adipogenic genes (Table 4). In multiple regression analysis, the relationship of SAT chemerin with insulin sensitivity (β=0.39, P<0.0001) as well as with SAT *IRS1* (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, the dietary intervention program resulted in a reduction in body weight of 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 a reduction in serum chemerin of 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 related with insulin sensitivity (r=−0.44, P=0.017), overall. An inverse association with the change in insulin sensitivity means that the higher the increase in M value, the higher the 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).

Table 3 Correlations between serum chemerin and other estimated parameters.

	Entire study group (*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.
AlAt, alanine aminotransferase; hsCRP, high-sensitive C-reactive protein; M, insulin sensitivity; SAT, s.c. adipose tissue.

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Table 4 Correlations between SAT *RARRES2* and other estimated parameters.

	Entire study group (n = 128)	**Normal weight** (n = 44)	**Overweight** (n = 44)	**Obese** (n = 40)
SAT *RARRES2*				
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.
M, insulin sensitivity.

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. $\Delta M_{ffm} > +3.298$ 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 a 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, 9, 10). Positive correlations between serum chemerin and parameters of obesity in populations with different fat mass and varying BMI were also reported in other studies (8, 9, 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 a positive correlation with BMI in a population with a wide range of BMI (9). However, these authors examined subjects older than those 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.,

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).

	Before (n = 30)	**After** (n = 30)
BMI (kg/m^2)	34.4 ± 2.32	30.3 ± 2.30[a]
Waist circumference (cm)	109 ± 8.9	99.9 ± 8.4[a]
% body fat	41.2 ± 4.3	36.3 ± 5.4[a]
Fasting plasma glucose (mg/dL)	88.7 ± 4.1	86.6 ± 6.3
Fasting serum insulin (μIU/mL)	14.9 ± 4.1	11.4 ± 3.0[a]
M (mg/kg ffm/min)	6.06 ± 2.71	7.89 ± 2.71[a]
Cholesterol (mg/dL)	194 ± 26.5	175 ± 31.2[a]
Triglycerides (mg/dL)	114 ± 58.9	93.1 ± 58.2
HDL-cholesterol (mg/dL)	51.8 ± 9.2	48.1 ± 9.3[a]
LDL-cholesterol (mg/dL)	126 ± 31.3	112 ± 30.8
hsCRP (mg/L)	1.40 ± 0.85	0.98 ± 0.90[a]
AlAt (U/L)	34.2 ± 8.9	19.0 ± 8.2[a]
Serum chemerin (ng/mL)	228 ± 37.0	211 ± 36.2[a]
SAT *RARRES2* (A.U.)	0.87 ± 0.37	0.91 ± 0.34

[a]$P < 0.05$ for the difference after vs before 12-week dietary intervention (n = 30).
AlAt, alanine aminotransferase; ffm, fat-free mass; hsCRP, high-sensitive C-reactive protein; M, insulin sensitivity.

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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 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 observed (17, 18). A positive relationship between SAT chemerin and insulin resistance has been observed (18), but the lack of any such correlation has also been described (4).

Based on our investigations, it appears that chemerin acting locally in SAT exerts a positive effect on whole-body insulin sensitivity, although the exact causality cannot be established. It could be due to its positive influence on adipogenesis, which results in proper AT function. In agreement with our suspicion, there are experimental studies which indicate that chemerin expression and secretion increase dramatically with adipogenesis, and that 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 that 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 the 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 lowest expression of genes associated with adipocyte differentiation and maturation in comparison with other study groups. Thus, we propose SAT chemerin as a biomarker of metabolically healthy SAT, because it may reflect preserved insulin action and adipogenesis in SAT. The fact that a 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 with 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 a metabolically unhealthy group with morbid obesity and with metabolic syndrome (25). 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 in parallel with a worsening of glucose tolerance status (26). Indeed, we observed a reduction in serum chemerin after weight loss with a 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 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 (27), 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

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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 (28). These findings may potentially explain the 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. An inverse correlation between circulating chemerin and SAT chemerin expression in humans with a wide range of obesity was also found by other investigators (17). However, in this study, differences in serum and SAT expression between controls and subjects with obesity were not presented (17). 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 previously mentioned 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 (29), it is possible that the liver might significantly contribute to systemic levels of chemerin. It has also been shown that chemerin levels were similar in portal vein blood and systemic venous blood, and significantly elevated in hepatic vein blood (30). Insufficiency of SAT results in an ectopic accumulation of fat, and in consequence leads to lipotoxicity and the development of insulin resistance. Liver affected by fat accumulation and insulin resistance could be responsible for increases in systemic chemerin. Chemerin mRNA was induced in the liver in a rodent model of obesity (31). Recent studies have reported elevated serum or hepatic expression levels of chemerin in NAFLD and found positive correlations with NAFLD active score or hepatic inflammation (12, 32, 33). We observed positive correlations of serum chemerin with AlAt and fetuin A, which are predictors of liver fat (34, 35), 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 (36).

However, one cannot exclude VAT as a source of increased serum chemerin concentration in obesity, as it has also been 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 by Svennson *et al*., chemerin secretion from VAT was marginally higher than from s.c. depot over 24 h; however, the pattern of chemerin secretion was very similar in both depots (37). 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 a group of obese women with normal glucose tolerance, as well as in a 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 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 that could be perceived as prejudicing the impartiality of the research reported.

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