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Molecules and Cells

Differential Patterns of Serum Concentration and Adipose Tissue Expression of Chemerin in Obesity: Adipose Depot Specificity and Gender Dimorphism

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Chemerin, a recognized chemoattractant, is expressed in adipose tissue and plays a role in adipocytes differentiation and metabolism. Gender- and adipose tissue-specific differences in human chemerin expression have not been well characterized. Therefore, these differences were assessed in the present study. The body mass index (BMI) and the circulating levels of chemerin and other inflammatory, adiposity and insulin resistance markers were assessed in female and male adults of varying degree of obesity. Chemerin mRNA expression was also measured in paired subcutaneous and visceral adipose tissue samples obtained from a subset of the study subjects. Serum chemerin concentrations correlated positively with BMI and serum leptin levels and negatively with high density lipoprotein (HDL)-cholesterol levels. No correlation was found between serum chemerin concentrations and fasting glucose, total cholesterol, low density lipoprotein (LDL)-cholesterol, triglycerides, insulin, C-reactive protein or adiponectin. Similarly, no relation was observed with the homeostasis model assessment for insulin resistance (HOMA-IR) values. Gender- and adipose tissue-specific differences were observed in chemerin mRNA expression levels, with expression significantly higher in women than men and in subcutaneous than visceral adipose tissue. Interestingly, we found a significant negative correlation between circulating chemerin levels and chemerin mRNA expression in subcutaneous fat. Among the subjects studied, circulating chemerin levels were associated with obesity markers but not with markers of insulin resistance. At the tissue level, fat depot-specific differential regulation of chemerin mRNA expression might contribute to the distinctive roles of subcutaneous vs. visceral adipose tissue in human obesity.

INTRODUCTION

Obesity plays a central role in the pathogenesis of several conditions, including insulin resistance, type 2 diabetes mellitus (T2DM), hypertension, atherosclerosis and coronary artery disease (CAD) (Hamdy et al., 2006).

Chemerin, a recently discovered circulating chemokine, exerts its actions through cell surface receptors termed chemokine-like receptor 1 (CMKLR1), G protein-coupled receptor 1 (GPR1), or chemokine (C-C motif) receptor-like 2 (CCRL2) (Barnea et al., 2008; Cash et al., 2008; Wittamer et al., 2003; Zabel et al., 2008). Later studies characterized chemerin as an adipokine with a potential role in regulating adipocyte development *in vitro* and metabolic functions, such as glucose and lipid metabolism, in adipose tissue (Goralski et al., 2007). Chemerin has been shown to affect insulin signaling in 3T3-L1 adipocytes (Kralisch et al., 2009; Takahashi et al., 2008). Compared with lean animals, chemerin gene expression is elevated in the adipose tissue of obese animals, and expression levels are markedly elevated during the differentiation of 3T3-L1 cells into mature adipocytes (Bozaoglu et al., 2007). Moreover, chemerin has been suggested to play a role in mediating the long-term beneficial effects of bariatric surgery (Ress et al., 2010). Because chemerin seems to play several roles in both inflammation and metabolism, it has been proposed as a link between chronic inflammation, obesity, and obesity-related comorbidities (Ernst and Sinal, 2010; Yang et al., 2010).

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The existence of intrinsic, fat depot-specific functional differences in both humans and animal models has been strongly supported by the differential gene expression of adipokines, such as leptin (Montague et al., 1997; 1998; Van Harmelen et al., 1998), plasminogen activator inhibitor-1 (Alessi et al., 1997), interleukin-6 (Fried et al., 1998), retinol-binding protein-4 (RBP4), and adiponectin (Samaras et al., 2010). In contrast, differences in the expression of human chemerin in subcutaneous vs. visceral fat, or potential gender-related differences in the circulating and adipose tissue-specific expression of chemerin, have not been well characterized. In this study, we examined the circulating concentrations of serum chemerin and the subcutaneous and visceral adipose tissue expression of chemerin mRNA in adult Saudi men and women. We determined whether gender- or adipose tissue-specific differences in chemerin expression exist and whether circulating or mRNA expression levels of chemerin are related to markers of inflammation, adiposity or insulin resistance.

MATERIALS AND METHODS

Study population

Study subjects included people undergoing elective abdominal surgery (n = 97, 63 females) for cholecystectomy or weight reduction and 28 age- and sex-matched healthy controls. The subjects' ages ranged from 17 to 71 years; all were free of acute inflammation, infection or malignancy. Sixteen subjects had T2DM, fifteen of whom were being treated by diet alone and one with metformin and insulin. All subjects had a stable weight with no fluctuations of > 2% for at least 2 months prior to surgery. This study was approved by the Institutional Review Board and performed at the Obesity Research Center of King Saud University, Riyadh, Saudi Arabia. Written informed consent was obtained from all participants.

Prior to surgery, all patients provided medical history and underwent a physical examination. Weight (in kilograms) was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured using a stadiometer to the nearest centimeter, and body mass index (BMI) was calculated (weight/height squared, and is reported in kilograms per square meter). The subjects' BMIs ranged from 19.5 to 66.8 kg/m^2.

After overnight fasting, blood samples were obtained, and sera and plasma were stored at -80°C until analytical measurements were performed. Paired subcutaneous and visceral fat samples were obtained from 23 subjects (14 females) during the surgical procedure. Subcutaneous fat (approximately 50 g) was cut from the port sites of the laparoscopic procedures in the anterior abdominal wall. In addition, visceral fat (approximately 100 g) was cut from the greater omentum of the abdominal adipose tissue. All tissues were immediately frozen in liquid nitrogen and stored at -80°C.

Measurement of biochemical parameters and insulin sensitivity

Serum levels of glucose, triglycerides, total cholesterol, and HDL-cholesterol were determined using the Dimension Xpand plus autoanalyzer (Siemens Healthcare Diagnostics, USA). Serum levels of LDL-cholesterol were calculated using Friedewald's equation (Friedewald et al., 1972). Plasma insulin levels were determined by electrochemiluminescence using a Cobas e411 immunoanalyzer (Roche, USA). HOMA-IR was determined for each subject according to the following equation: fasting glucose (mmol/l) × fasting insulin (mU/ml)/22.5 (Matthews et al., 1985).

Measurement of circulating adipokines and inflammatory markers

Commercially available ELISA kits were used to measure the serum concentrations of leptin, adiponectin, chemerin (Millipore corporation, USA), and high-sensitivity C Reactive Protein (hsCRP, Immunodiagnostik AG, Germany) according to the manufacturers' recommended protocols (leptin intra-assay CV 4.9%, inter-assay CV 8.6%; adiponectin intra-assay CV 7.4%, inter-assay CV 8.4%; chemerin intra-assay CV 5.0%, inter-assay CV 6.0%; and hsCRP intra-assay CV 6.0%, inter-assay CV 11.6%).

RNA extraction and real-time PCR

RNA was extracted from adipose tissue samples using the RNeasy Lipid Tissue kit (Qiagen, Germany). Extraction was followed by a DNase digestion step to remove contaminating genomic DNA. RNA was quantitated using the Nanodrop ND-1000 Spectrophotometer (LabTech, UK). The quality of the RNA was further evaluated by agarose gel electrophoresis and visual inspection of the 28S and 18S ribosomal RNA bands. From each sample, 300 ng of RNA was reverse-transcribed using the High Capacity RNA-to-cDNA Kit (Applied Biosystems, USA) according to the manufacturer's instructions. For real-time PCR, 0.2 μl of the synthesized cDNA (equivalent to 3 ng of input RNA) was loaded per well in a 96-well plate, and the relative levels of human chemerin mRNA expression were detected in duplicate using the inventoried TaqMan primer and probe set, HS 00161209_g1 (Applied Biosystems, USA). Samples were incubated at 95°C for 10 min to allow for initial denaturation, followed by 40 PCR cycles, with each cycle consisting of 95°C for 15 s and 60°C for 1 min. The 7500 Real-Time PCR system was used (Applied Biosystems). Data were obtained as Ct values (the cycle number at which logarithmic PCR plots cross a calculated threshold line) according to the manufacturer's guidelines. Ct values were used to determine Δ CT: Ct of the target gene (chemerin) - Ct of the housekeeping gene (GAPDH). Human chemerin mRNA expression levels were normalized to GAPDH and calculated using the ΔΔ CT method (Livak and Schmittgen, 2001). For the ΔΔ CT calculation to be valid, the amplification efficiencies of the target and endogenous control must be nearly equal. A validation experiment using a dilution of the cDNA samples was performed, and the results of the amplification of chemerin and GAPDH (Δ CT) were plotted vs. the log of the input amount of total RNA. The absolute value for the slope of the resulting semi-log regression line was < 0.1. Therefore, the amplification efficiencies of target and endogenous control genes were equal, and the ΔΔ CT calculation could be used for the relative quantification of chemerin mRNA expression.

Statistical analysis

Data are shown as means ± SD unless otherwise stated. Prior to statistical analysis, logarithmic transformation of the non-normally distributed parameters was performed to approximate a normal distribution. *P* values < 0.05 were considered statistically significant. Associations between variables were evaluated by Pearson correlation analysis and the results presented as correlation coefficients (r).

RESULTS

Anthropometric and biochemical parameters

Serum chemerin concentrations were not significantly different between men (77.83 ± 32.82 ng/ml) and women (82.5 ± 22.06 ng/ml); however, as expected, there was a significant, gender-

Table 1. Clinical and biochemical characteristics of the study subjects

Variable	Value	Male	Female	*P* value[a]
Number (n)	125	52	73	
Age (years)	35.79 ± 11.26	33.29 ± 12.66	37.58 ± 13.24	0.07
BMI (kg/m^2)	36.64 ± 12.63	35.36 ± 13.66	37.54 ± 11.64	0.34
Fasting glucose (mmol/L)	5.8 ± 1.43	5.87 ± 1.66	5.75 ± 1.7	0.69
Insulin (mIU/L)	14.67 ± 13.64	16.73 ± 18	13.18 ± 7.9	0.14
HOMA-IR	4.07 ± 5.32	4.51 ± 6.48	3.27 ± 2.07	0.13
Total cholesterol (mmol/L)	4.7 ± 0.98	4.63 ± 0.95	4.75 ± 1.01	0.5
LDL-cholesterol (mmol/L)	2.91 ± 0.79	2.87 ± 0.81	2.93 ± 0.81	0.67
HDL-cholesterol (mmol/L)	1.18 ± 0.3	1.14 ± 0.25	1.22 ± 0.31	0.13
Triglycerides (mmol/L)	1.27 ± 0.61	1.35 ± 0.7	1.21 ± 0.58	0.21
hsCRP (μg/mL)	13.8 ± 15.54	12.71 ± 13.78	14.77 ± 15.08	0.48
Leptin (ng/mL)	33.41 ± 27.07	20.08 ± 19.35	43.46 ± 24.51	**0.00**
Adiponectin (μg/mL)	7.52 ± 3.85	6.72 ± 2.78	8.1 ± 4.23	**0.04**
Chemerin (ng/mL)	80.55 ± 25.4	77.83 ± 32.82	82.5 ± 22.06	0.35

Data are presented as mean ± standard deviation. BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein. [a]*P* value resulting from a male vs. female comparison of the values for each of the measured parameters. Values < 0.05 (bolded) are considered statistically significant.

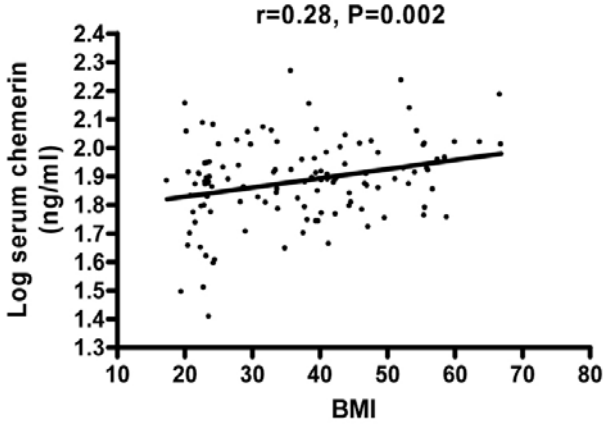

Fig. 1. Correlation of serum chemerin concentration and body mass index (BMI; Kg/m^2) (n = 125)

Table 2. Linear regression analysis of biochemical variables associated with serum chemerin levels (n = 125)

Variable	Pearson correlation (r)	*P* value[a]
Fasting glucose	-0.03	0.74
Total cholesterol	0.00	0.94
LDL-cholesterol	0.01	0.88
HDL-cholesterol	-0.19	**0.04**
Triglycerides	0.09	0.33
Insulin	0.11	0.22
HOMA-IR	0.09	0.32
CRP	0.17	0.11
Leptin	0.22	**0.03**
Adiponectin	-0.15	0.1

[a] *P* values < 0.05 (bolded) are considered statistically significant.

related difference in the circulating levels of leptin and adiponectin, with higher levels detected in women (Table 1). There was a significant positive correlation between serum chemerin concentrations and BMI (Fig. 1), as well as between circulating chemerin and leptin concentrations (Table 2). A negative correlation was observed between serum chemerin concentrations and HDL-cholesterol levels. In contrast, no correlation was found between serum chemerin concentrations and fasting glucose, total cholesterol, LDL-cholesterol, triglycerides, insulin, hsCRP or adiponectin or between chemerin concentrations and HOMA-IR values (Table 2).

Visceral and subcutaneous chemerin mRNA expression

Chemerin mRNA expression was significantly higher in subcutaneous than visceral adipose tissue (Fig. 2). Subdivision of the subjects according to their gender revealed a gender-related difference in chemerin mRNA expression, with a significantly higher level of expression in female than male subcutaneous adipose tissue. In addition, within each gender, higher chemerin mRNA expression was detected in subcutaneous than visceral adipose tissue (Fig. 3).

The serum chemerin concentration correlated negatively with chemerin mRNA expression in subcutaneous fat; however, a negative trend, which did not reach statistical significance, was found between circulating chemerin levels and chemerin mRNA expression in visceral fat (Figs. 4A and 4B, respectively; n = 23).

An analysis of chemerin mRNA expression in visceral fat in relation to other clinical and biochemical variables revealed no significant correlations (data not shown). By contrast, chemerin mRNA expression in subcutaneous fat correlated negatively with circulating triglyceride concentrations (r = -0.47, P = 0.027) and positively correlated with circulating adiponectin (r = 0.43, P = 0.045).

DISCUSSION

In obesity, altered secretion of various adipokines is closely linked to metabolic changes that ultimately result in associated-

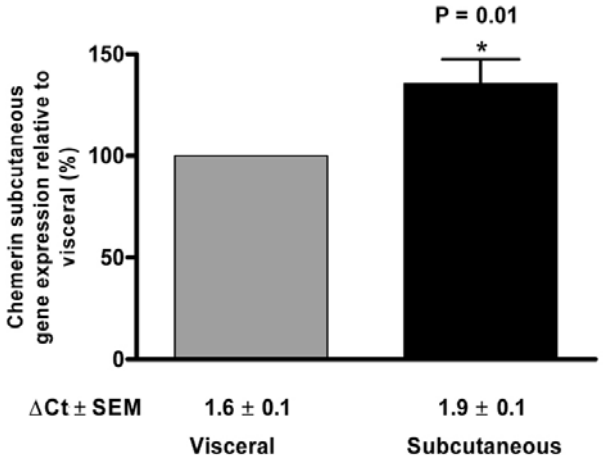

Fig. 2. Chemerin mRNA expression in visceral and subcutaneous adipose tissue. Chemerin mRNA expression levels in subcutaneous fat are shown relative to those in visceral fat (n = 23), which was arbitrarily assigned a value of 100%.

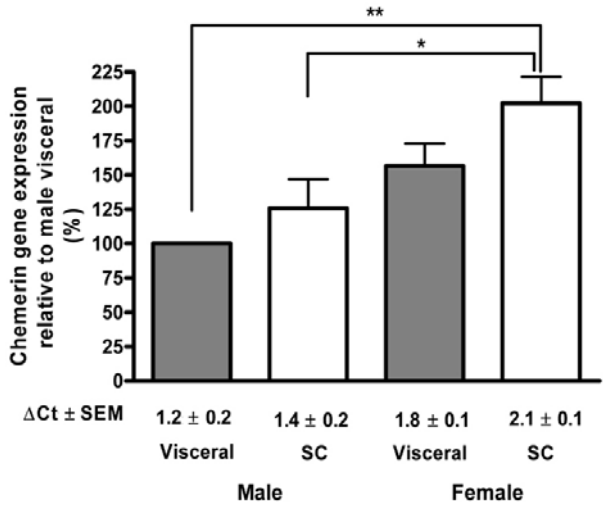

Fig. 3. Chemerin mRNA expression in visceral and subcutaneous adipose tissue in males and females. Chemerin mRNA expression levels in male subcutaneous fat (n = 9), female visceral fat (n = 14), and female subcutaneous fat (n = 14) were compared to male visceral fat (n = 9), which was arbitrarily assigned a value of 100% (*P < 0.05, **P < 0.01).

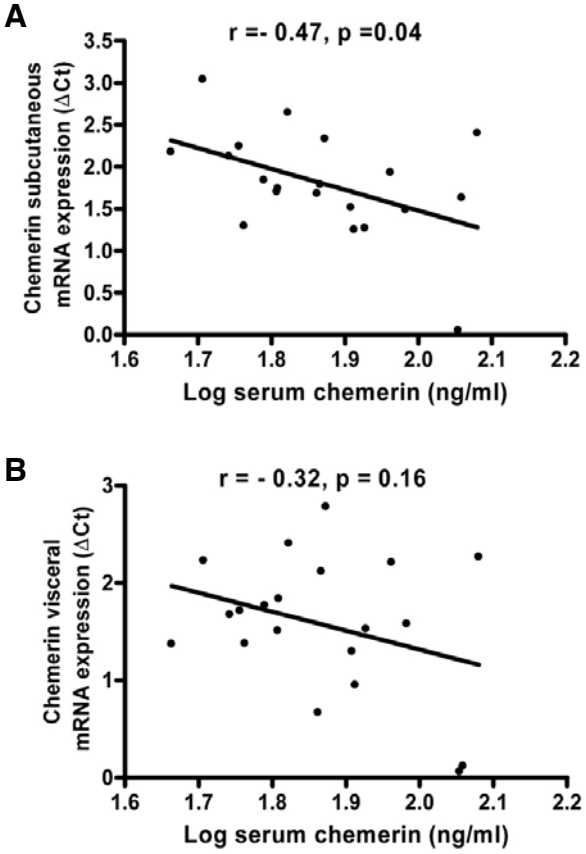

Fig. 4. (A) Correlation of serum chemerin concentration and chemerin mRNA expression in subcutaneous fat depots. (B) Correlation of serum chemerin concentration and chemerin mRNA expression in visceral fat depots.

metabolic diseases. Several recent studies have demonstrated that circulating chemerin levels are elevated in both obese humans and obese/diabetic experimental animals and are positively correlated with various aspects of metabolic syndrome (Bozaoglu et al., 2007; Ernst and Sinal, 2010; Sell et al., 2009). The administration of exogenous chemerin to obese/diabetic (but not normoglycemic) mice resulted in exacerbated glucose intolerance, lower serum insulin levels, and decreased tissue glucose uptake (Ernst et al., 2010). Such data corroborate the notion that chemerin contributes to the undesirable changes that eventually result in obesity comorbidities. Nevertheless, the role played by chemerin has been a matter of controversy and has not yet been clearly elucidated, particularly in humans.

We investigated the relations between circulating levels of chemerin and various markers of inflammation, adiposity and insulin resistance. In addition, chemerin mRNA expression levels were measured in paired adipose tissue subcutaneous and visceral samples obtained from adult Saudi subjects with a wide range of obesity and insulin sensitivities.

This work emphasizes the above-mentioned potential link between chemerin expression and the pathogenesis of obesity in a well characterized adult population. Similar to previous reports, we found a positive, statistically significant correlation between circulating chemerin and BMI. In contrast to these same reports, we found no association between circulating chemerin and insulin resistance markers (Bozaoglu et al., 2007; Sell et al., 2010; Tan et al., 2009). The role of chemerin in insulin sensitivity/resistance is still a matter of controversy. Although chemerin is among the adipokines that are present at statistically lower levels in "insulin sensitive-" obese individuals than "insulin resistant-" obese individuals (Klöting et al., 2010), several *in vitro* studies have produced conflicting results. Some research groups have shown that in the 3T3-L1 cell line, increased chemerin expression resulted in increased insulin-induced IRS1 tyrosine phosphorylation and glucose uptake (Takahashi et al., 2008); however, other groups have demonstrated the reverse in the same cell line, where chemerin significantly decreased insulin stimulated-glucose uptake, indicating insulin resistance (Kralisch et al., 2009). Furthermore, there have been reports with conflicting results where chemerin expression was examined as a function of insulin sensitivity in

response to thiazolidinediones (TZDs). Whereas some studies suggested that using these insulin sensitizers was associated with elevated chemerin expression (Roh et al., 2007), others reported the opposite (Vernochet et al., 2009; 2010). Our results suggest that among the studied group of adult Saudis, chemerin is not a clear marker of insulin resistance. Nevertheless, owing to our small sample size, the potential for type 2 errors and false negatives should be taken into account, particularly when the aim is to determine the exact role of chemerin in the Saudi population. It is also possible that the association reported previously between chemerin levels and the degree of insulin resistance was because both elevated levels of chemerin and insulin resistance are detected in morbidly obese individuals. Thus, a causal or mechanistic relation might not necessarily exist and further investigations are required to clarify this issue.

Differential expression of chemerin mRNA in adipose tissue depots was reported in both wild-type and ob/ob mice. In the wild-type mice, chemerin and CMKLR1 were highly expressed in white adipose relative to brown adipose tissue; however, in the ob/ob mice, chemerin mRNA expression was upregulated in brown but not white fat and hence, chemerin levels became similar in the two types of adipose tissue. Chemerin mRNA levels in paired adipose tissue samples obtained from patients with polycystic ovarian syndrome (PCOS) were recently published (Tan et al., 2009). A significant increase in chemerin mRNA expression in subcutaneous and omental adipose tissue in PCOS compared to normal subjects was reported, although no fat-depot-specific differences in chemerin mRNA levels were detected in either group. In contrast, our data support the possibility that chemerin expression may vary by location in human white adipose tissue (subcutaneous vs. visceral fat depots). Although further testing is required, we hypothesize that subcutaneous fat depots are the major source of circulating chemerin in humans, as we were able to demonstrate significantly higher chemerin mRNA expression in subcutaneous than in visceral adipose tissue. In addition, the present work demonstrates that circulating levels of chemerin were negatively correlated with chemerin mRNA expression in subcutaneous adipose tissue; however, this correlation did not exist in visceral adipose tissue. Further analysis is required to determine whether the negative correlation observed specifically in subcutaneous adipose tissue results from a negative feedback loop, whereby elevated circulating chemerin levels inhibit chemerin mRNA expression.

Our analysis of the paired samples of adipose tissue revealed that the chemerin mRNA expression level in visceral adipose tissue was inversely related to that in subcutaneous adipose tissue for several subjects (data not shown). This finding further implies different roles of chemerin in subcutaneous and visceral adipose tissue.

Interestingly, in the present study, a gender-related difference in chemerin mRNA expression was detected. Women express more chemerin mRNA than men in both subcutaneous and visceral adipose tissues. Further investigation is indeed required to explain this finding.

Although most of the published data support a proinflammatory role for chemerin (Ernst and Sinal, 2010; Kralisch et al., 2009; Parlee et al., 2010), some studies have related chemerin expression to anti-inflammatory actions (Cash et al., 2008; Luangsay et al., 2009). These conflicting results can potentially be explained by the presence of multiple chemerin-derived peptides. Whereas peptides derived from cleavage of the biologically inactive pro-chemerin by serine proteases exert chemotactic/proinflammatory activity (Wittamer et al., 2005), cysteine protease-derived peptides have anti-inflammatory effects (Cash et al., 2008). In the present study, results from the ELISA assay revealed a positive association between the circulating levels of chemerin and leptin; however, the same was not true for chemerin mRNA expression levels in adipose tissue (both subcutaneous and visceral) and circulating leptin. Intriguingly, chemerin mRNA expression in subcutaneous adipose tissue was positively correlated with the circulating levels of adiponectin (an anti-inflammatory adipokine) and negatively correlated with the circulating levels of triglycerides (an atherogenic marker). This finding raises the question of whether chemerin can play dual roles, exerting both inflammatory and anti-inflammatory effects. Determining the major chemerin cleavage products present both in the circulation and locally in different fat depots will be crucial for gaining a better understanding of the *in vivo* roles of chemerin. The commercially-available chemerin ELISA kits measure total chemerin levels. Development of antibodies specific for different chemerin cleavage products would seem necessary to clearly elucidate the role of chemerin and/or its cleavage products in obesity-related diseases. Alternatively, a proteomics approach could be undertaken in an effort to accurately determine the exact molecular form(s) of chemerin present in the circulation and in various tissues, including different fat depots.

In this study, we demonstrated that serum chemerin concentrations are positively correlated with BMI and with circulating leptin levels but negatively correlated with HDL-cholesterol levels and the expression levels of chemerin mRNA in subcutaneous adipose tissue. Both gender- and fat depot-related differences in chemerin mRNA expression were demonstrated. The latter might represent an additional factor that partially explains the differential contribution of subcutaneous and visceral fat depots to human obesity.

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