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Effects of weight loss and exercise on chemerin serum concentrations and adipose tissue expression in human obesity

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ABSTRACT

Chemerin is a chemoattractant adipokine that regulates adipogenesis and may induce insulin resistance. Chemerin serum concentrations are elevated in obese, insulin-resistant, and inflammatory states in vivo. Here we investigate the role of omental (OM) and subcutaneous (SC) adipose tissue *chemerin* and *CMKLR1* messenger RNA (mRNA) expression in human obesity. In addition, we test the hypothesis that changes in chemerin serum concentrations are primarily associated with reduced body fat mass in the context of 3 weight loss intervention studies. Chemerin serum concentration was measured in 740 individuals in a cross-sectional (n = 629) study including a subgroup (n = 161) for which OM and SC *chemerin* mRNA expression has been analyzed as well as in 3 interventions including 12 weeks of exercise (n = 60), 6 months of calorie-restricted diet (n = 19) studies, and 12 months after bariatric surgery (n = 32). *Chemerin* mRNA is significantly higher expressed in adipose tissue of patients with type 2 diabetes mellitus and correlates with circulating chemerin, body mass index (BMI), percentage body fat, C-reactive protein, homeostasis model assessment of insulin resistance, and glucose infusion rate in euglycemic-hyperinsulinemic clamps. *CMKLR1* mRNA expression was not significantly different between the 2 fat depots. Obesity surgery–induced weight loss causes a significant reduction on both OM and SC *chemerin* expression. All interventions led to significantly reduced chemerin serum concentrations. Decreased chemerin serum concentrations significantly correlate with improved glucose infusion rate and reduced C-reactive protein levels independently of changes in BMI. Insulin resistance and inflammation are BMI-independent predictors of elevated chemerin serum concentrations. Reduced chemerin expression and serum concentration may contribute to improved insulin sensitivity and subclinical inflammation beyond significant weight loss.

Authors' contributions: RC performed experiments, analyzed data, and wrote manuscript; MR performed experiments; NK performed experiments and analyzed data; AO performed exercise intervention; GF performed diet intervention and experiments, and analyzed data; MK performed experiments and researched data; MRS performed bariatric surgery studies; ES performed surgery studies; TL researched data and edited manuscript; MD performed weight loss studies; MF performed experiments and contributed to discussion; MS edited manuscript; MB designed studies, performed experiments, analyzed data, and wrote manuscript.

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1. Introduction

Chemerin is a chemoattractant protein secreted from adipose tissue and the liver that regulates adipocyte differentiation as well as chemotaxis and activation of dendritic cells and macrophages [1]. Chemerin modulates activation of dendritic cells and macrophages through the G protein–coupled receptors CMKLR1 (ChemR23), GPR1, and CCRL2 [2-4]. In addition, chemerin has been shown to regulate adipocyte differentiation in an autocrine/paracrine manner and modulates the expression of adipocyte genes involved in glucose and lipid metabolism [5,6]. In skeletal muscle cells, chemerin induces insulin resistance by an impairment of insulin receptor signaling and glucose uptake [7]. Moreover, Becker et al [8] recently demonstrated that expression of human chemerin induces insulin resistance in the skeletal muscle of low-density lipoprotein receptor knockout mice on high-fat diet. Administration of exogenous chemerin deteriorates glucose tolerance; lowers serum insulin levels; and decreases tissue glucose uptake in *ob/ob*, *db/db*, and diet-induced obese but not lean and normoglycemic mice [9].

In humans, circulating chemerin was shown to be associated with multiple components of the metabolic syndrome, including body mass index (BMI), triglycerides, high-density lipoprotein cholesterol, and hypertension [5,10,11], and also with systemic markers of inflammation, such as high-sensitivity C-reactive protein (hsCrP), interleukin-6, and tumor necrosis factor–α [1,12,13]. In morbidly obese patients undergoing bariatric surgery, sustained reduction of chemerin serum concentrations was associated with weight loss and improvement of metabolic parameters [11,14]. We have recently shown that higher chemerin serum concentrations are already detectable in prediabetic states [15] and may reflect adipose tissue dysfunction independently of body fat mass [16]. Collectively, human and rodent data indicate that chemerin influences glucose homeostasis and may contribute to the link between increased adipose tissue mass/fatty liver disease and obesity-related metabolic and inflammatory diseases. Here we test the hypothesis that reduced chemerin messenger RNA (mRNA) expression in human adipose tissue contributes to reduced circulating chemerin concentrations after bariatric surgery. In parallel to chemerin, we measured mRNA expression of its receptor *CMKLR1* in human omental (OM) and subcutaneous (SC) adipose tissue. In addition, we aim to dissect whether changes in chemerin serum concentrations are primarily associated with improved insulin sensitivity or reduced body fat mass in the context of 3 weight loss intervention studies (6 months of hypocaloric diet, 12 months after bariatric surgery, 12 weeks of exercise program).

2. Methods

2.1. Subjects

We included 5 different cohorts with a total number of 740 individuals in our study of chemerin serum concentration and adipose tissue mRNA expression. In the first cohort (n = 468), we investigated chemerin serum concentrations in relation to measures of obesity and glucose metabolism in a cross-sectional study (cohort 1). In another cross-sectional study (cohort 2), we investigated chemerin mRNA expression in paired OM and SC adipose tissue samples in addition to chemerin serum concentrations (n = 161). In a third study (cohort 3), we investigated circulating chemerin in response to a 12-week intensive exercise intervention in 60 individuals with different degrees of glucose tolerance. In addition, we measured circulating chemerin before and 6 months after a calorie-restricted diet study (cohort 4, n = 19) and before and 12 months after bariatric surgery (cohort 5, n = 32). Individuals of all cohorts fulfilled the following inclusion criteria: (1) absence of any acute or chronic inflammatory disease as determined by a leukocyte count greater than 7.0×10^9 cells/L, CrP greater than 5.0 mg/dL, or clinical signs of infection; (2) undetectable antibodies against glutamic acid decarboxylase; (3) no medical history of hypertension, that is, systolic blood pressure was less than 140 mm Hg and diastolic blood pressure was less than 85 mm Hg; (4) no clinical evidence of either cardiovascular or peripheral artery disease; (5) no thyroid dysfunction; (6) no alcohol or drug abuse; and (7) no pregnancy. All study protocols have been approved by the ethics committee of the University of Leipzig. All participants gave written informed consent before taking part in the study. The 3 cohorts had the following specific characteristics.

2.1.1. Cohort 1

A total of 468 white men (n = 220) and women (n = 248) have been consecutively recruited in the context of a study on insulin resistance at the Department of Medicine, University of Leipzig, to represent a wide range of obesity, insulin sensitivity, and glucose tolerance. The age ranged from 19 to 80 years, and BMI was from 17.1 to 79.1 kg/m^2. The study included 290 patients with type 2 diabetes mellitus (T2D) and 178 normal glucose tolerant (NGT) controls (Supplementary Table 1). From these individuals, we selected 58 that could be matched for age, sex, and BMI into subgroups of NGT and T2D (Supplementary Table 2).

2.1.2. Cohort 2

Adipose tissue *chemerin* and *CMKLR1* mRNA expression was investigated in previously described 161 donors of paired OM and SC adipose tissue samples [17] who underwent abdominal surgery for cholecystectomy, weight reduction surgery, abdominal injuries, or explorative laparotomy. From these individuals, we selected 32 that could be matched for age, sex, and BMI into subgroups of NGT and T2D. All subjects had a *stable weight*, defined as the absence of fluctuations of more than 2% of body weight for at least 3 months before surgery. Adipose tissue was immediately frozen in liquid nitrogen after explantation. Histologic analyses and measurement of macrophage count in adipose tissue were performed as previously described [18].

2.1.3. Cohort 3

Sixty subjects were divided into groups of NGT (n = 20; 9 male, 11 female), impaired glucose tolerance (IGT; n = 20; 9 male, 11 female), and T2D (n = 20; 11 male, 9 female) on the basis of a 75-g oral glucose tolerance test (OGTT) according to the American Diabetes Association criteria [19] (Supplementary

Table 3). Subjects with NGT were defined by a fasting plasma glucose less than 6.0 mmol/L and a 120-minute plasma glucose less than 7.8 mmol/L. Subjects with IGT were defined by a fasting plasma glucose less than 6.0 mmol/L, and a 120-minute plasma glucose greater than 7.8 mmol/L and less than 11.1 mmol/L. Subjects with T2D were defined by a fasting plasma glucose greater than 7.0 mmol/L and/or a 120-minute OGTT glucose greater than 11.1 mmol/L. These 60 individuals were enrolled in 60 minutes of supervised physical training sessions 3 days per week as described previously [20]. In brief, each training session included 20 minutes of biking or running, 20 minutes of swimming, and 20 minutes of warming up/cooling down periods. All subjects completed a graded bicycle ergometer test to volitional exhaustion and had maximal oxygen uptake measured with an automated open circuit gas analysis system at baseline and after 4 and 12 weeks of training. The highest oxygen uptake per minute reached was defined as the *maximal oxygen uptake*, and subjects subsequently trained at their individual *submaximal heart rate* defined as 70% to 80% of the individual maximal heart rate during the bicycle ergometer test. At baseline and after 4 and 12 weeks of training (48 hours after the last training session), blood samples were obtained in the fasting state; and measurements of anthropometric parameters were performed.

2.1.4. Cohort 4

2.1.4.1. *Six-month hypocaloric diet study*. Nineteen white obese volunteers (15 female, 4 male) attending the Obesity Outpatients Clinic at the University of Leipzig Medical Department were recruited (Supplementary Table 4). Patients underwent a clinical assessment including medical history, physical examination, dual x-ray absorptiometry scan analysis, comorbidity evaluation, as well as nutritional interviews performed by a multidisciplinary consultation team. In addition to the general exclusion criteria, patients with T2D and volunteers with any concomitant medication have been excluded from the study. Weight loss was achieved over a period of 6 months by a diet providing a daily energy deficit of 1200 kcal/d. Diet adherence was monitored by daily food intake protocols.

2.1.5. Cohort 5

2.1.5.1. *Bariatric surgery study*. Thirty-two white obese volunteers (22 female, 10 male) participated in a prospective weight loss study before and 12 months after gastric sleeve resection or Roux-en-Y gastric bypass (Supplementary Table 4). In a subgroup of 14 (10 female, 4 male) patients, OM and SC adipose tissue biopsies were obtained in the context of a 2-step bariatric surgery strategy with gastric sleeve resection as the first step and a Roux-en-Y gastric bypass as second-step operation. The baseline BMI in this subgroup was 64.1 ± 9.5 kg/m^2, and the BMI 12 months after bariatric surgery was 48.3 ± 7.3 kg/m^2.

2.2. Measurement of body fat content, glucose metabolism, and insulin sensitivity

Body mass index was calculated as weight divided by squared height. Hip circumference was measured over the buttocks; waist circumference was measured at the midpoint between the lower ribs and iliac crest. Percentage body fat was measured by dual x-ray absorptiometry. In cohort 2, abdominal visceral and SC fat areas were calculated using computed tomography (CT) scans at the level of L4-L5 in the cohort of paired visceral and SC adipose tissue donors. Three days before the OGTT, patients documented a high-carbohydrate diet in diet protocols. The OGTT was performed after an overnight fast with 75-g standardized glucose solution (Glucodex Solution 75 g; Merieux, Montreal, Canada). Venous blood samples were taken at 0, 60, and 120 minutes for measurements of plasma glucose concentrations. Insulin sensitivity was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR) index or with the euglycemic-hyperinsulinemic clamp method as described previously [21].

2.3. Analyses of blood samples

All baseline blood samples were collected between 8:00 AM and 10:00 AM after an overnight fast. Plasma insulin was measured with an enzyme immunometric assay for the IMMULITE automated analyzer (Diagnostic Products, Los Angeles, CA). Serum hsCrP, adiponectin, interleukin-6, leptin, and monocyte chemoattractant protein–1 were measured as previously described [16]. Serum chemerin was measured by an enzyme-linked immunosorbent assay (Biovendor, Heidelberg, Germany).

2.4. *Chemerin* and *CMKLR1* mRNA expression studies

Human *chemerin* and *CMKLR1* mRNA expression was measured by quantitative real-time reverse transcriptase polymerase chain reaction (PCR) in a fluorescent temperature cycler using the TaqMan assay, and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems, Darmstadt, Germany). Total RNA was isolated using TRIzol (Life Technologies, Grand Island, NY), and 1 μg RNA was reverse transcribed with standard reagents (Life Technologies). From each reverse transcriptase PCR, 2 μL was amplified in a 26-μL PCR using the Brilliant SYBR Green QPCR Core Reagent Kit from Stratagene (La Jolla, CA) according to the manufacturer's instructions. Samples were incubated in the ABI PRISM 7000 sequence detector for an initial denaturation at 95 °C for 10 minutes, followed by 40 PCR cycles, each cycle consisting of 95°C for 15 seconds, 60°C for 1 minute, and 72°C for 1 minute. The following primers were used: for chemerin, 5′-GGAAGAAACCCGAGTGC AAAG-3′ and 5′-TGATGCAGGCCAGGCATT-3′; for *CMKLR*, 5′-CTCCCAATCCATATCACCTA-3′ and 5′-GCAGAGGAAGAA GGTAATGA-3′. SYBR Green fluorescence emissions were monitored after each cycle. Human *chemerin* and *CMKLR1* mRNA expression was calculated relative to the mRNA expression of 18s ribosomal RNA (rRNA), determined by a premixed assay on demand for 18s rRNA (Applied Biosystems). Amplification of specific transcripts was confirmed by melting curve profiles (cooling the sample to 68°C and heating slowly to 95°C with measurement of fluorescence) at the end of each PCR. The specificity of the PCR was further verified by subjecting the amplification products to agarose gel electrophoresis.

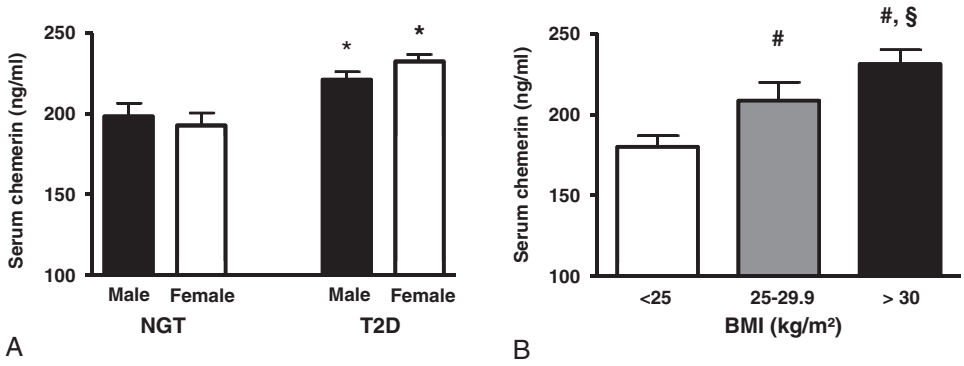

Fig. 1 – Chemerin serum concentration in NGT individuals and patients with T2D. A, Circulating chemerin in men (n = 93) and women (n = 85) with NGT and in men (n = 127) and women (n = 163) with T2D. B, Chemerin serum concentrations in lean (BMI <25 kg/m^2; n = 42), overweight (BMI >25–29.9 kg/m^2; n = 58), and obese (BMI >30 kg/m^{2}n = 78) NGT subjects. *P < .05 adjusted for BMI compared with NGT within sexes. #P < .05 compared with BMI less than 24.9 kg/m^2. §P < .05 compared with BMI 25 to 29.9 kg/m^2.

2.5. *Statistical analyses*

Data are shown as means ± SD unless stated otherwise. Before statistical analysis, nonnormally distributed parameters were logarithmically transformed to approximate a normal distribution. The following statistical tests were used: paired Student test, χ^2 test, and Pearson simple correlation. Linear relationships were assessed by least square regression analysis. Statistical analysis was performed using SPSS version 12.0 (Chicago, IL). *P* values < .05 were considered to be statistically significant.

3. Results

3.1. *Chemerin serum concentration in obesity and T2D*

Anthropometric and metabolic characteristics of 468 individuals included in the cross-sectional study (cohort 1) are summarized in Supplementary Table 1. Circulating chemerin was not different between men and women with NGT and T2D (Fig. 1A). Chemerin serum concentration was approximately 15% higher in individuals with T2D as compared with NGT (Fig. 1A) (*P* < .05). In age-, sex-, and BMI-matched subgroups of

Table 1 – Univariate correlations (Spearman) between chemerin serum concentration and chemerin mRNA expression in OM and SC adipose tissue and measures of obesity, insulin sensitivity, and parameters of inflammation

Cohorts (n)	Serum chemerin (baseline)		OM *chemerin* mRNA		SC *chemerin* mRNA	
	Cohorts 1-5 (n = 740)		Cohort 2 (n = 161)		Cohort 2 (n = 161)	
	r (BMI-adjusted *r*)	*P* value (BMI-adjusted *P*)	*r* (BMI-adjusted *r*)	*P* value (BMI-adjusted *P*)	*r* (BMI-adjusted *r*)	*P* value (BMI-adjusted *P*)
Serum chemerin	–	–	0.33	<.01	0.16	.03
Age	0.33 (0.21)	<.0001 (<.01)	0.21 (0.04)	<.01 (NS)	0.12 (0.06)	NS (NS)
Sex			0.03	NS	0.06	NS
BMI	0.35 (-)	<.0001 (–)	0.33 (-)	<.01 (–)	0.24 (-)	<.01 (–)
% Body fat	0.39 (0.05)	<.0001 (NS)	0.25 (0.13)	<.01 (.06)	0.22 (0.1)	<.01 (.09)
Waist circumference	0.21 (0.05)	.003 (NS)	0.18 (0.1)	.02 (NS)	0.11 (0.08)	NS (NS)
Hip circumference	0.17 (0.03)	.02 (NS)	0.09 (0.03)	NS (NS)	0.06 (0.04)	NS (NS)
Fasting plasma glucose	0.19 (0.07)	.007 (NS)	0.1 (0.03)	NS (NS)	0.07 (0.05)	NS (NS)
HbA_{1c}	0.14 (0.05)	.009 (NS)	0.2 (0.08)	.02 (NS)	0.1 (0.04)	NS (NS)
Fasting plasma insulin	0.2 (0.09)	.005 (NS)	0.38 (0.19)	<.01 (<.05)	0.24 (0.08)	<.01 (NS)
HOMA-IR	0.22 (0.13)	.002 (.08)	0.35 (0.22)	<.01 (<.01)	0.19 (0.09)	<.01 (NS)
GIR	0.26 (0.17)	.001 (.01)	0.41 (0.27)	<.01 (<.01)	0.26 (0.11)	<.01 (NS)
Triglycerides	0.2 (0.05)	.006 (NS)	0.1 (0.02)	NS (NS)	0.31 (0.19)	<.01 (<.01)
hsCrP	0.38 (0.25)	<.0001 (<.01)	0.38 (0.26)	<.01 (<.01)	0.35 (0.22)	<.01 (<.01)
Creatinine	0.25 (0.16)	<.0001 (.02)	0.04 (0.02)	NS (NS)	0.01 (0.01)	NS (NS)
Mean adipocyte size	0.18 (0.15)	.008 (<.05)	0.32 (0.18)	<.01 (<.05)	0.27 (0.15)	<.01 (<.05)
% Macrophages in adipose tissue	0.15 (0.14)	.03 (<.05)	0.35 (0.23)	<.01 (<.01)	0.07 (0.02)	NS (NS)

In brackets: *r* and *P* value adjusted for BMI. *r* indicates Spearman correlation coefficient; NS, not significant.

NGT and T2D, we confirmed significantly higher chemerin serum concentrations in T2D vs NGT (Supplementary Table 2). In addition, we found significantly increasing chemerin serum concentrations from lean to overweight and obese individuals with NGT (Fig. 1B). In patients with T2D (n = 290), serum chemerin concentration correlates with BMI ($r = 0.27$, $P < .01$). In 740 patients (representing the baseline chemerin serum concentrations in cohorts 1-5) with a wide range of age, BMI, glucose tolerance, and insulin sensitivity, we found significant relationships between circulating chemerin and parameters of (abdominal) obesity, glucose and lipid metabolism, insulin sensitivity, and adipose tissue biology (Table 1). Univariate regression analyses revealed significant correlations between chemerin serum concentration and age, BMI, percentage body fat, waist and hip circumferences, fasting plasma glucose, hemoglobin A_{1c} (HbA_{1c}), fasting plasma insulin, insulin sensitivity as determined by HOMA-IR and/or the glucose infusion rate (GIR) during the steady state of an euglycemic-hyperinsulinemic clamp, triglycerides, CrP, creatinine, as well as adipocyte size and number of macrophages in visceral adipose tissue (Table 1). Correlations between circulating chemerin and age, GIR, CrP, creatinine, and parameters of adipose tissue biology remained significant even after adjusting for BMI (Table 1).

3.2. *Chemerin and CMKLR1 mRNA expression in OM and SC adipose tissue*

We investigated *chemerin* and *CMKLR1* mRNA expression in visceral and SC adipose tissue in parallel with chemerin serum concentrations in 161 previously described individuals [17], who have been classified into lean and predominantly subcutaneously or viscerally obese on the basis of abdominal visceral and SC fat area measurements using CT or magnetic resonance imaging scans at the level of L4-L5 (cohort 2). In NGT individuals, *chemerin* expression was not different between the OM and SC fat depot, whereas in patients with T2D, *chemerin* mRNA expression was significantly higher in OM compared with SC fat (Fig. 2A). Moreover, *chemerin* expression of both fat depots was approximately 1.4-fold higher in the T2D compared with the NGT group (Fig. 2A). In age-, sex-, and BMI-matched subgroups of NGT (n = 16) and T2D (n = 16), we confirmed significantly higher chemerin mRNA expression in both fat depots in T2D (OM: 13.7 ± 0.8; SC:

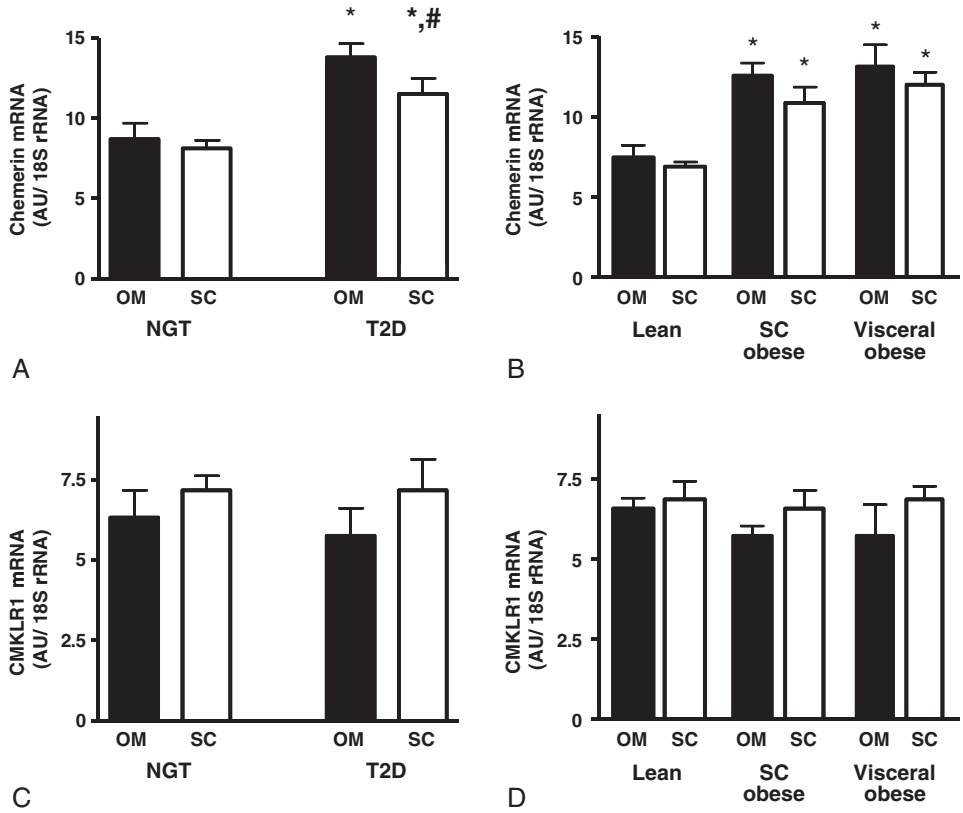

Fig. 2 – *Chemerin* and *CMKLR1* mRNA expression in human OM and SC adipose tissue. A, *Chemerin* mRNA expression in the 2 different fat depots in NGT individuals (n = 116) and patients with T2D (n = 45). *$P < .05$ adjusted for BMI compared with the NGT group. #$P < .05$ for the difference between OM and SC. B, Omental and SC *chemerin* mRNA expression in lean, viscerally obese, and subcutaneously obese individuals. Abdominal visceral and SC fat areas were calculated using CT scans at the level of L4-L5 in the cohort of paired visceral and SC adipose tissue donors. *Visceral obesity* was defined as greater than 0.4 ratio of visceral to SC fat area as determined by CT scan. C, *CMKLR1* mRNA expression in the 2 different fat depots in NGT individuals (n = 116) and patients with T2D (n = 45). D, Omental and SC *CMKLR1*mRNA expression in lean, viscerally obese, and subcutaneously obese individuals. Data are means ± SEM. *$P < .05$ compared with the lean group.

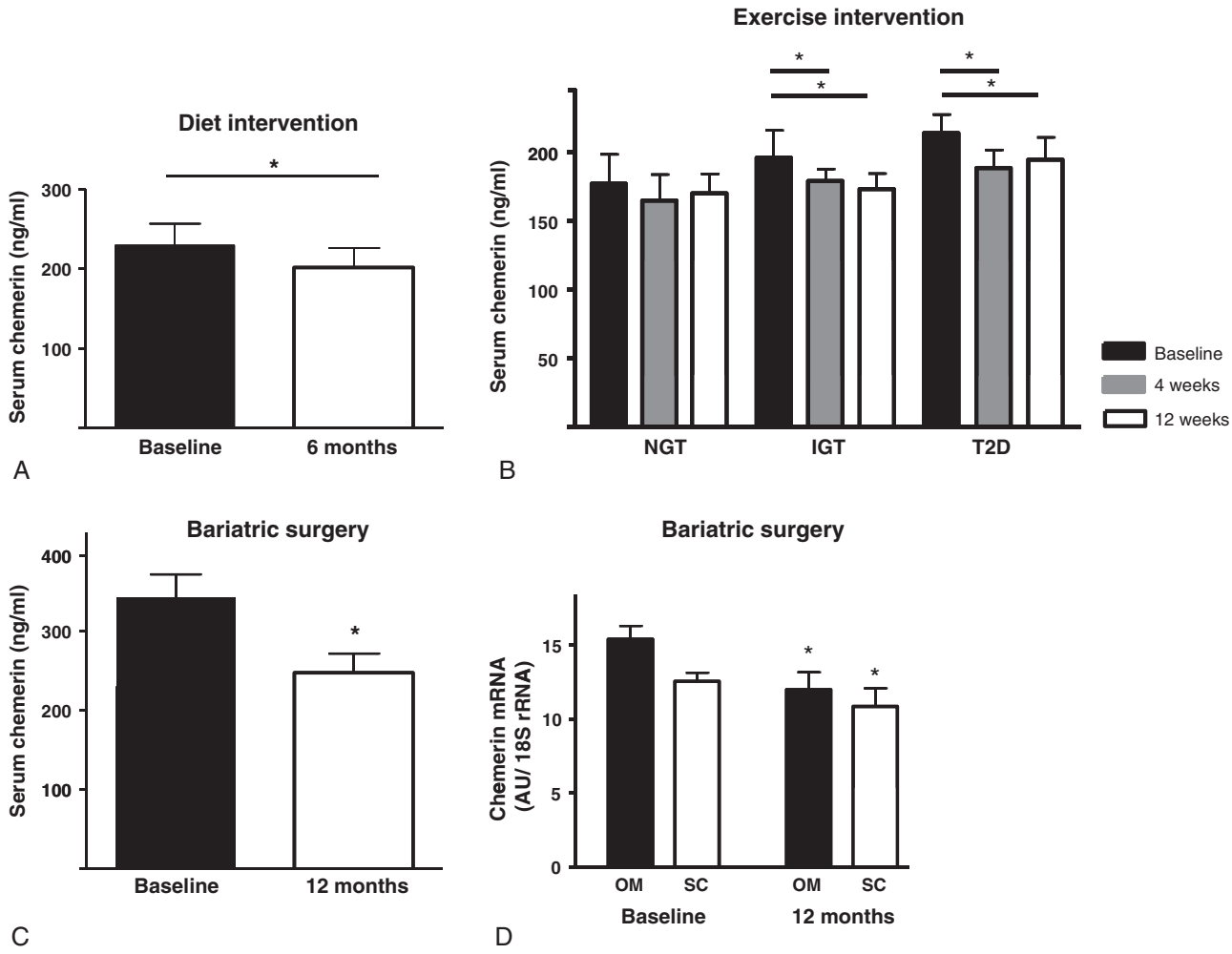

Fig. 3 – Changes of chemerin serum concentration in response to different weight loss interventions. Effect of 6-month calorie-restricted diet (A), 12-week exercise intervention (B), and bariatric surgery on chemerin serum concentration (C) and chemerin mRNA expression (D) in OM adipose tissue. Data are means ± SEM. *P < .05 adjusted for BMI compared with baseline.

12.2 ± 0.4 AU/18s rRNA) compared with NGT (OM: 9.1 ± 0.4; SC: 8.4 ± 0.5 AU/18s rRNA). Lean individuals showed significantly lower *chemerin* expression in visceral and SC adipose tissue compared with both subcutaneously and viscerally obese patients (Fig. 2B). Noteworthy, *chemerin* expression (OM and SC) was not different between these 2 obesity subclasses. We found significant correlations of OM and SC *chemerin* mRNA expression with chemerin serum concentration and parameters of obesity, glycemic control, insulin sensitivity, inflammation, and adipose tissue biology (Table 1). The correlations between OM *chemerin* mRNA expression and mean adipocyte size, number of macrophages in adipose tissue, CrP, GIR, HOMA-IR, and fasting plasma insulin remained significant after adjusting for BMI (Table 1). Subcutaneous *chemerin* mRNA expression only correlates with adipocyte size, CrP, and serum triglyceride levels independently of BMI (Table 1). In contrast to chemerin, *CMKLR1* mRNA expression was not significantly different between OM and SC adipose tissue in both NGT and T2D individuals (Fig. 2C). There was a tendency for lower *CMKLR1* mRNA expression in OM, but not SC, adipose tissue in obese compared with lean individuals (Fig. 2D).

3.3. *Changes in chemerin serum concentrations in response to different weight loss interventions*

Serum chemerin concentrations are significantly reduced after a 6-month hypocaloric diet intervention (cohort 4) (Fig. 3A). Multivariate linear regression analyses identified changes in BMI, insulin sensitivity, and circulating CrP as significant predictors of changes in circulating chemerin. Interestingly, both improved GIR and CrP are independent of changes in body weight associated with changes in chemerin levels (Table 2).

We further sought to dissect the effects of weight loss vs improved chronic glycemia and insulin sensitivity on reduced chemerin serum concentrations in the context of a 12-week exercise intervention (cohort 3). Sixty white men and women completed a 12-week training program and were studied after being divided into subjects with NGT (n = 20), IGT (n = 20), or T2D (n = 20) as previously described [20] (Supplementary Table 2). Chemerin serum concentration was significantly higher in individuals with IGT and T2D compared with NGT (Fig. 3B). However, these differences were entirely explained by significant differences in BMI (Supplementary Table 2). The training

Table 2 – Multivariate linear regression analysis of changes in different parameters as predictors of reduced chemerin serum concentration in response to 3 different interventions (12-week exercise program, 6-month hypocaloric diet, 12 months after bariatric surgery)

	Δ Chemerin serum concentration		
	Exercise intervention	Hypocaloric diet	Bariatric surgery
	β Coefficient (P value)	β Coefficient (P value)	β Coefficient (P value)
Model 1			
Age	0.05 (.89)	0.04 (.85)	0.09 (.83)
Sex	0.03 (.92)	0.06 (.81)	0.04 (.94)
Δ BMI	0.12 (.28)	**0.31 (<.001)**	**0.42 (<.001)**
Model 2			
Age	0.06 (.79)	0.03 (.91)	0.08 (.81)
Sex	0.04 (.9)	0.04 (.88)	0.02 (.9)
Δ BMI	0.07 (.75)	**0.25 (<.01)**	**0.36 (<.001)**
Δ GIR	**−0.34 (<.001)**	**−0.16 (<.05)**	**−0.24 (<.01)**
Model 3			
Age	0.04 (.83)	0.05 (.86)	0.06 (.88)
Sex	0.04 (.86)	0.03 (.91)	0.04 (.92)
Δ BMI	0.1 (.31)	**0.18 (<.05)**	**0.25 (<.001)**
Δ hsCrP	**0.41 (<.001)**	**0.32 (<.001)**	**0.29 (<.001)**

Significant correlations are shown in bold. Δ indicates change of parameter.

effect was confirmed by a significant improvement in VO_2 max in all groups. Twelve weeks of physical training resulted in significantly improved insulin sensitivity, HbA_{1c}, and circulating CrP in the IGT and T2D groups despite the fact that BMI was not significantly changed. Chemerin serum concentrations significantly decreased by 7% to 10% in IGT and T2D subjects after 4 and 12 weeks of the training program (Fig. 3B). In the NGT group, there was only a trend for lower chemerin serum concentrations in response to the exercise intervention (Fig. 3B).

Interestingly, multivariate linear regression analyses demonstrate similar to the data of the diet intervention study that changes in GIR and CrP are independent of the BMI dynamic related to changes in chemerin serum concentrations (Table 2).

In a third intervention study (cohort 5), we aimed to analyze changes in both chemerin serum concentration and adipose tissue mRNA expression before and 12 moths after obesity surgery. Patients with morbid obesity have significantly higher chemerin serum concentrations than all other investigated subgroups (Fig. 3C). We found approximately 25% lower chemerin serum concentrations 12 months after significant weight loss (Table 2) induced by bariatric surgery (Fig. 3C). In parallel, we found in a subgroup of patients (n = 14) undergoing a 2-step bariatric surgery strategy significantly reduced OM and SC *chemerin* mRNA expression (Fig. 3D). Multivariate regression analyses of changes in BMI, GIR, and CrP as predictors for changes in chemerin serum concentrations demonstrate in analogy with the other intervention studies that changes in GIR and CrP are significantly associated with changes in chemerin beyond the effects of body weight changes (Table 2).

4. Discussion

Chemerin is a hepatoadipokine that has been shown to induce insulin resistance in skeletal muscle in vivo [7], suggesting an important role in the cross talk between adipose tissue, liver, and skeletal muscle. High serum chemerin concentrations in humans are associated with higher BMI [5] and traits of the metabolic syndrome including high plasma glucose and triglycerides, low high-density lipoprotein cholesterol, and elevated blood pressure [10], as well as insulin-resistant states including women with polycystic ovary syndrome [22]. Moreover, Sell et al [11] recently demonstrated that chemerin plasma concentrations are significantly elevated in morbidly obese patients and can be reduced by weight loss induced after obesity surgery. Hyperinsulinemia was shown to upregulate circulating chemerin, whereas metformin decreases elevated chemerin serum concentrations [22]. However, it is still controversial whether elevated circulating chemerin levels are primarily due to obesity [5,11], insulin resistance [11,16,22], hyperglycemia [15], or inflammatory response [12]. We therefore addressed this controversy by measuring the dynamic of chemerin serum concentrations in 3 independent intervention studies. In all studies, we found significant BMI-independent correlations between reduced chemerin levels and improved insulin sensitivity as well as decreased hsCrP serum concentrations, suggesting that both insulin resistance and chronic inflammation may contribute to elevated chemerin serum concentrations.

Two studies, a 6-month hypocaloric diet study (cohort 4) and a bariatric surgery follow-up study (cohort 5), were designed to test the effects on circulating chemerin achieved by significant weight loss. We show here for the first time that moderate weight loss 6 months after a calorie-restricted diet significantly decreases chemerin serum concentrations. Decrease in chemerin levels can be explained by significant correlations with changes in BMI, insulin sensitivity, and hsCrP. Our data from the bariatric surgery study confirm previous findings that circulating chemerin is significantly elevated in morbidly obese patients [11] and can be significantly reduced by drastic reduction in BMI [11,14]. However, multivariate linear regression analyses demonstrated that, independently of these BMI changes, reduced hsCrP serum concentrations and improved insulin sensitivity significantly predict changes in circulating chemerin. The third intervention (cohort 3), a 12-week exercise program, provided the opportunity to directly assess the effects of improved insulin sensitivity and reduced circulating inflammatory markers such as hsCrP in the absence of significant changes in BMI. We show here that improved CrP levels and GIR in euglycemic-hyperinsulinemic clamps correlated with reduced chemerin levels despite a stable BMI. This relationship between chemerin serum concentration and insulin sensitivity could explain previous data showing that circulating chemerin is significantly lower in insulin-sensitive compared with insulin-resistant obese subjects who have been matched for age, sex, BMI, and body fat mass [16]. Our data are further supported by a previous study demonstrating that insulin increases chemerin release in vivo [22].

In accordance with recent reports [11,15,23,24], but in contrast to others [5,12], we found significantly higher chemerin serum concentrations in patients with T2D. In age-, sex-, and BMI-matched subgroups of NGT and T2D, we demonstrate that these differences were independent of BMI. We show here that elevated chemerin serum concentrations may be due to significantly increased OM and SC adipose tissue *chemerin* mRNA expression. Increased *chemerin* expression in patients with T2D was more pronounced in the OM compared with the abdominal SC fat depot, suggesting a fat depot–specific regulation of *chemerin* expression. We found significantly higher *chemerin* expression in OM compared with SC in T2D patients, whereas no *chemerin* expression differences were observed in the NGT group. This result closely reflects data obtained in polycystic ovary syndrome patients, which show a similar upregulation of OM adipose tissue *chemerin* expression, whereas healthy controls had no fat depot–specific expression of chemerin [22]. Noteworthy, we did not find significant differences in the mRNA expression of the chemerin receptor *CMKLR1* between different fat depots, individuals with NGT and T2D, or lean and obese adipose tissue donors, suggesting that changes in adipose tissue function are primarily associated with changes in *chemerin* and not *CMKLR1* expression. Adipose tissue chemerin expression studies further revealed significant associations between the chemerin mRNA and adipocyte size as well as number of macrophages in OM adipose tissue. The latter correlation has been described earlier [11] and may link adipose tissue inflammation or dysfunction [25] to increased chemerin expression. A potential link between adipose tissue inflammation and elevated chemerin secretion is further supported by the finding that tumor necrosis factor–α increases chemerin release by adipocytes [7]. The correlation between increased adipocyte size, in both the OM and SC depots, and chemerin expression and serum concentrations may mechanistically link adipocyte hypertrophy and subsequent insulin resistance to increased chemerin serum concentrations. However, it is difficult to establish a causality chain whether adipocyte hypertrophy or adipose tissue inflammation causes increased chemerin serum concentrations or whether increased chemerin expression causes adipose tissue inflammation with subsequent insulin resistance and chronic inflammation. Chemerin may play a role at an early stage of adipose tissue inflammation because chemerin as a chemoattractant protein may contribute to recruitment of macrophages into adipose tissue [1-4]. In addition, chemerin may modulate adipose tissue function directly because chemerin has been shown to regulate adipocyte differentiation and expression of adipocyte genes involved in glucose and lipid metabolism [5,6]. We further show that significantly increased *chemerin* mRNA expression and circulating chemerin in patients with morbid obesity can be significantly reduced by significant weight loss in response to obesity surgery. These data further suggest that adipose tissue chemerin production significantly contributes to circulating chemerin concentrations. This notion is supported by significant correlations between adipose tissue *chemerin* mRNA expression and chemerin serum concentrations. It has been previously shown that serum chemerin is higher in peripheral venous blood compared with portal vein blood [12] and that chemerin secretion from the liver might significantly contribute to systemic chemerin levels [26]. However, our study design does not allow investigating the role of hepatic chemerin secretion in the observed correlations between chemerin and measures of obesity, insulin sensitivity, glucose metabolism, and inflammation.

In conclusion, we show here that impaired insulin sensitivity and circulating parameters of inflammation are BMI-independent predictors of elevated chemerin serum concentrations in obesity and obesity-associated diseases. Our data support an important role of chemerin in the initiation of adipose tissue inflammation and dysfunction and suggest that reduced adipose tissue chemerin expression may contribute to improved insulin sensitivity and subclinical inflammation beyond significant weight loss.

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Conflict of Interest

The authors do not have any conflict of interest related to this manuscript. They disclose any financial conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.metabol.2011.10.008.

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