Chemerin Correlates with Markers for Fatty Liver in Morbidly Obese Patients and Strongly Decreases after Weight Loss Induced by Bariatric Surgery

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Context: Chemerin is a new adipokine involved in *in vitro* adipogenesis and insulin resistance and associates with body mass index (BMI) *in vivo*.

Objective: We investigated the role of chemerin in morbid obesity, associated metabolic diseases (insulin resistance, hepatic diseases), and postsurgery-induced weight loss.

Setting: This was a prospective study performed at a university hospital.

Subjects: Subjects included 60 obese female patients (BMI 50.0 $\pm$ 1.0 kg/m^{-2}) being candidates for gastric bypass.

Study Design: Patients were examined before and 3, 6, and 12 months after surgery. In 27 patients, chemerin was measured after 2 yr.

Main Outcome: Outcomes included chemerin, anthropometric parameters, homeostasis model assessment for insulin resistance index (HOMA-IR), cholesterol, high-density lipoprotein, triglycerides, C-reactive protein, adipokines at all time points; and liver histology and macrophage content in fat at baseline.

Results: Chemerin was substantially elevated in obese patients compared with nonobese persons (353.8 $\pm$ 18.0 *vs.* 191 $\pm$ 14 ng/ml, $P < 0.001$). Preoperatively, chemerin concentrations correlated positively with BMI, C-reactive protein, IL-6, HOMA-IR, and the amount of omental macrophages and negatively with high-density lipoprotein levels. Baseline chemerin was elevated in patients with a significant activity score for nonalcoholic fatty liver disease, portal inflammation, fibrosis, and fibroinflammation. After surgery, chemerin decreased significantly to 253.0 $\pm$ 14.9 ng/ml after 1 yr and pursued its decrease in patients studied for 2 yr. After surgery, chemerin concentrations positively correlated with triglycerides. The strong decrease of chemerin in the 3 months after surgery was associated with the decrease in HOMA-IR and blood glucose.

Conclusions: Chemerin concentrations are elevated in morbidly obese patients and correlated with insulin resistance and markers of liver pathology. Chemerin plasma concentrations decreased after bariatric surgery. This study suggests that chemerin might mediate metabolic alterations in obesity, drastically improving after gastric bypass. **(*J Clin Endocrinol Metab* 95: 2892–2896, 2010)**

Obesity is one of the most serious health hazards and frequently accompanied by metabolic disturbances (1). Increased adipose tissue mass, especially in the visceral compartment, is characterized by altered metabolic and endocrine function leading to an increased secretion of proinflammatory adipokines. These secreted molecules may be factors underlying the association between increased body fat and metabolic complications such as insulin resistance in peripheral organs or ectopic fat accumulation. Chemerin is a recently described adipokine

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Abbreviations: BMI, Body mass index; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; hsCRP, high sensitive C-reactive protein; IR, insulin resistance; MANOVA, multivariate analysis of variance; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis.

(2, 3). Chemerin is elevated in adipose tissue of diabetic *Psammomys obesus* compared with controls (2). In humans chemerin plasma concentrations correlate with body mass index (BMI) (2) and are elevated in patients with the metabolic syndrome being associated with blood glucose, high-density lipoprotein (HDL)-cholesterol, triglycerides, and blood pressure (4). The relationships between chemerin and the metabolic state in morbid obesity and the effect of weight loss have not yet been studied. Therefore, this study aimed at analyzing chemerin levels in morbidly obese patients before and during surgery-induced weight loss and the relationship between chemerin concentration, anthropometric measurements, biochemical parameters, markers of inflammation, and hepatosteatosis in a longitudinal study at baseline and 12–24 months after surgery.

Subjects and Methods

Subjects and study design

The study enrolled 60 unrelated obese patients recruited at the Pitié-Salpêtrière-Hospital (Paris, France) (5). Patients met the criteria for obesity surgery, *i.e.* BMI 40 kg/m^2 or greater or 35 kg/m^{-2} or greater with at least one comorbidity. A Roux-en-Y-gastric bypass was performed in most patients (n = 59) excepted in one (sleeve gastrectomy). Evaluation before surgery included medical history and physical, nutritional, metabolic, cardiopulmonary, and psychological assessments. The subject's weight was stable for at least 3 months before operation. Subjects did not demonstrate evidence of acute or chronic inflammatory disease, infectious diseases, viral infection, cancer, or known alcohol consumption (>20 g/d). Various clinical and biological parameters were assessed at baseline and at 3, 6, and 12 months after surgery. In a subgroup of 27 patients, data collection was continued for 24 months after surgery. According to the criteria of fasting glycemia greater than 7 mM or the use of an antidiabetic drug, 21 subjects were type 2 diabetics who were treated with metformin and fibrates or statins. Five subjects were treated with insulin. Clinical studies were approved by the French Ethics Committee. All patients signed an informed written consent. In all obese subjects, fat mass and lean body mass were determined by dual-energy x-ray absorptiometry (DEXA; GE Lunar Prodigy, Madison, WI). Control patients were recruited at Hannover Medical School, Germany.

Laboratory tests

Venous blood samples were collected after an overnight fast to determine leptin, adiponectin, IL-6, high sensitive C-reactive protein (hsCRP) and chemerin. Plasma glucose, triglycerides, total cholesterol, and HDL-cholesterol levels were measured enzymatically. Serum insulin concentrations were determined with Bi-INSULIN immunoradiometric assay (CisBio International, Gif-sur-Yvette, France). Leptin and adiponectin were determined using RIA (Linco Research, St. Louis, MO). Serum IL-6 was measured by an ultrasensitive ELISA (Quantikine US; R&D Systems, Abingdon, UK). Chemerin ELISA kits came from Biovendor (Heidelberg, Germany).

Liver histopathology

Major histopathological features were semiquantified according to previously published criteria (6, 7). The amount of steatosis, defined as the percentage of hepatocytes with fat droplets, was scored using the following scale: 0 (0–5%), 1 (5–33%), 2 (34–66%), and 3 (>67%). Foci of lobular inflammation were classified as 0 (none) and 1 (one to four foci) (averaged from three to four fields at ×20 magnification). Portal inflammation was evaluated by inflammatory infiltrate assessed at low magnification and scored 0 (none to minimal) and 1 (significant). Fibrosis was scored according to Brunt (6), as F0 (none), F1 (zone 3 perisinusoidal or portal fibrosis), and F2 (zone 3 perisinusoidal and periportal fibrosis without bridging). Presence of nonalcoholic steatohepatitis (NASH) was evaluated using the nonalcoholic fatty liver disease activity score (NAS) established by Kleiner (7), as the unweighted sum of scores of steatosis, lobular inflammation, and hepatocellular ballooning. NAS was classified as low (0–1) and significant (score 2–6). NASH was considered as certain for NAS of 5 or more. Severity of fibrosis and inflammation was assessed by creating a fibroinflammation score, which is the unweighted sum of elementary scores of fibrosis, portal inflammation, and lobular inflammation (8). The participants were subclassified as those with significant and mild or absent liver lesions.

Adipose tissue macrophage quantification

Paired biopsies of omental and sc adipose tissue were used to assess the number of infiltrating adipose tissue macrophages as described (9).

Insulin sensitivity calculation

Homeostasis model assessment (HOMA) for insulin resistance (IR) was determined in all patients except those treated with insulin by a mathematical transformation of fasting blood glucose and insulin measurements. This index has been validated in comparison with the euglycemic-hyperinsulinemic clamp and represents a useful index when studying morbidly obese individuals.

Statistical analyses

Data are expressed as means ± SEM. The Shapiro-Wilcoxon test was used to test the Gaussian distribution of biological parameters. All variables were log transformed to normalize their distribution before statistical analyses. Student's *t* test, ANOVA followed by *P* for linear trend posttest when appropriate, and χ^2 test for noncontinuous variables were used for comparison be-

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tween groups. Correlations were performed by Pearson. The kinetic evolution of the different parameters in the obese subjects was evaluated by multivariate analysis of variance (MANOVA). For adjustment (BMI, diabetic state), we applied a multiple linear regression modeling using least squares means. Statistical analyses were performed with JMP (SAS Institute Inc., Cary, NC) or Prism (GraphPad Software, San Diego, CA). $P < 0.05$ was considered significant.

Results

Chemerin concentrations at baseline and association with clinical parameters

Chemerin circulating concentrations were significantly increased in morbidly obese patients (354 ± 18 ng/ml for obese patients compared with 191 ± 14 ng/ml for 13 lean volunteers (BMI 21.5 ± 1.7 kg/m^{-2}). Chemerin concentrations in morbidly obese patients at baseline positively correlated with BMI, HOMA-IR, hs-CRP, IL-6, orosomucoid, and the amount of macrophages in omental adipose tissue, and negatively associated with HDL-cholesterol (Supplemental Table 1, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). After adjustment for BMI, chemerin concentrations remained associated with HDL-cholesterol, orosomucoid, and the amount of macrophages in omental fat. Importantly chemerin circulating concentrations were not different in diabetics (367 ± 32 ng/ml) and nondiabetics (347 ± 22 ng/ml).

Plasma chemerin concentrations associate with liver biomarkers and histopathology

Patients characterized by mild steatosis and the absence of lobular inflammation had slightly lower chemerin circulating concentrations and patients with a low activity score were characterized by significantly lower chemerin concentrations than patients with higher scores (Table 1). The three patients characterized by a NASH (NAS ≥ 5) had very high chemerin concentrations (487 ± 18 ng/ml). Furthermore, chemerin circulating concentrations significantly increase with the severity of liver fibrosis. Chemerin concentrations were highly associated with the presence of portal inflammation and fibroinflammation, a correlation that persisted after BMI adjustments, but that was not reflected by increased chemerin mRNA expression (data not shown). IL-6 was elevated only in patients with hepatosteatosis, whereas no liver marker was associated with leptin or adiponectin (data not shown).

Plasma chemerin concentrations and clinical parameters after surgery

Gastric surgery induced a significant reduction in weight and fat mass and caused a significant improvement of various clinical parameters as plasma glucose, insulin, HOMA, lipids, hsCRP, aminotransferase, γ-glutamyltransferase, orosomucoid, and fibrinogen (Supplemental Table 2). In parallel, leptin and IL-6 concentrations decreased, whereas adiponectin increased after surgery (Fig. 1, A–C).

TABLE 1. Liver histological characteristics of morbidly obese subjects classified according to severity and in relation to chemerin levels

	n	Chemerin (ng/ml)	*P* value	*P* value after adjustment for BMI
Steatosis				
Mild (0–2)	15	341 ± 36		
Significant (≥3)	29	393 ± 26	NS	NS
Lobular inflammation				
Absent (0)	24	355 ± 29		
Present (1–2)	20	400 ± 32	NS	NS
Activity (NAS)				
Low (0–1)	15	296 ± 33		
Significant (≥2)	28	410 ± 24	0.013	NS
Fibrosis				
Absent	16	310 ± 33		
Grade 1	18	393 ± 31		
Grade 2	10	449 ± 42	0.042	NS
Portal inflammation				
Absent	22	302 ± 26		
Present	22	449 ± 26	0.0002	0.032
Fibroinflammation (NAFIN)				
Mild (0–1)	17	294 ± 31		
Significant (≥2)	21	425 ± 28	0.0029	0.033

NS, Not significant; NAFIN, nonalcoholic fibroinflammation.

Chemerin concentrations significantly decreased in the first year after surgery (Fig. 1D). In a subgroup of 27 patients, chemerin concentrations still fall significantly between 1 and 2 yr after surgery (Fig. 1E). Strikingly, this decrease in chemerin circulating concentrations could be observed in the absence of a further change in BMI, fat mass, HOMA, leptin, adiponectin, and IL-6 (Supplemental Table 2).

After surgery, chemerin correlated with triglyceride levels also when adjusted for BMI (Supplemental Table 3). The decrease in chemerin concentrations until month 3 significantly correlated with a decrease in glucose and HOMA-IR. After 12 months after surgery, Δchemerin correlates with ΔHDL-cholesterol and ΔCRP.

Discussion

Bariatric surgery is the most effective treatment of morbid obesity leading to a reduction in many adipokines (10). Adiponectin that has insulin-sensitizing properties in liver and skeletal muscle is increased after bariatric surgery (11), whereas leptin and proinflammatory mediators like IL-6 and macrophage chemoattractant protein-1 decrease after bariatric surgery. We here show for the first time that chemerin plasma concentrations are significantly elevated

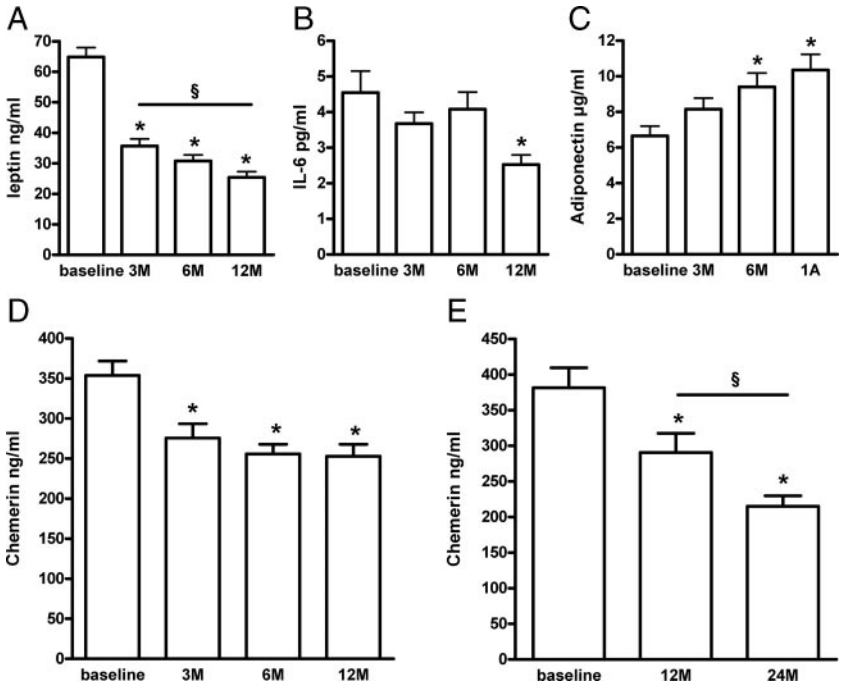

FIG. 1. Plasma concentrations of adipokines after obesity surgery. A–C, Levels of leptin, IL-6, and adiponectin were measured in all patients at baseline and 3–12 months after obesity surgery ($P < 0.01$ as evaluated by MANOVA). *, Significantly different from baseline; §, significantly different from designated values on *post hoc* tests. D and E, Chemerin plasma concentrations were determined in all patients at baseline and 3–12 months after bariatric surgery. In a subset of 27 patients, chemerin levels were measured at baseline and 12 and 24 months after surgery ($P < 0.01$ as evaluated by MANOVA). *, Significantly different from baseline; §, significantly different from designated values on *post hoc* tests.

in morbidly obese patients and that obesity surgery induces its drastic reduction. Chemerin fell most prominently in the first 3 months after surgery when weight and fat mass loss, improvement of insulin sensitivity, and inflammatory markers were the strongest. In subgroups of patients, chemerin still significantly decreases between 1 and 2 yr after surgery. This prolonged effect of surgery on chemerin levels occurred in the absence of reduction of BMI, fat mass, HOMA-IR, and other adipokines. Thus, this extended decrease of chemerin might be independent from a further reduced adipose tissue mass and improvement of insulin sensitivity.

Chemerin was shown to correlate with BMI and components of the metabolic syndrome in normal and overweight subjects (2, 4, 12). We show this correlation also in morbid obesity. A correlation with insulin sensitivity could be found in contrast to others in which no difference in chemerin concentrations between diabetic and control patients could be found (2, 12). Insulin increases the chemerin release *in vivo* (13). Interestingly, we here found a correlation between the insulin resistance index HOMA-IR and chemerin. Furthermore, circulating chemerin concentrations were associated with the amount of macrophages in omental adipose tissue. Because human macrophages do not secrete chemerin (14), it must be assumed that adipocytes and preadipocytes contribute to whole-body chemerin levels. Adipose tissue inflammation might play a role in chemerin secretion because TNFα increases the chemerin release by adipocytes (14).

Adipokines have been shown to be related to liver pathology and also in morbid obesity (15). Chemerin levels are significantly elevated in morbidly obese women characterized by a significant activity score for nonalcoholic fatty liver disease and fibroinflammation. We could not find changes in leptin or adiponectin in patients with liver pathology in this study, whereas higher IL-6 secretion could be shown to be associated with steatosis, corroborating other studies (16). The mechanism by which increased adipose tissue mass and the concomitant increase of adipokines might be involved in hepatosteatosis includes the infiltration of macrophages in adipose tissue (8, 17). We could observe an association of chemerin with the amount of macrophages in omental fat together with a relationship between chemerin and liver pathology. Thus, it might be speculated that chemerin could be a link between adipose tissue inflammation and liver pathology in obesity. This hypothesis needs to be tested in larger cohorts with liver histopathological characterization. Chemerin concentrations are higher in venous hepatic blood than portal venous blood (12), and hepatocytes express chemerin; thus, a contribution of liver to chemerin concentrations might be considered (18) and should be addressed in future studies.

In conclusion, chemerin plasma concentrations are significantly elevated in morbidly obese patients and are correlated to BMI, insulin resistance, adipose tissue inflammation, hepatosteatosis, and liver inflammation. Surgical obesity treatment inducing strong weight and fat mass loss decreases chemerin levels in a more prolonged way than other adipokines including leptin.

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References

1. **Bosello O, Zamboni M** 2000 Visceral obesity and metabolic syndrome. Obes Rev 1:47–56
2. **Bozaoglu K, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, Walder K, Segal D** 2007 Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinology 148:4687–4694
3. **Goralski KB, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, Parlee SD, Muruganandan S, Sinal CJ** 2007 Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. J Biol Chem 282:28175–28188
4. **Bozaoglu K, Segal D, Shields KA, Cummings N, Curran JE, Comuzzie AG, Mahaney MC, Rainwater DL, VandeBerg JL, MacCluer JW, Collier G, Blangero J, Walder K, Jowett JB** 2009 Chemerin is associated with metabolic syndrome phenotypes in a Mexican-American population. J Clin Endocrinol Metab 94:3085–3088
5. **Poitou C, Coussieu C, Rouault C, Coupaye M, Cancello R, Bedel JF, Gouillon M, Bouillot JL, Oppert JM, Basdevant A, Clément K** 2006 Serum amyloid A: a marker of adiposity-induced low-grade inflammation but not of metabolic status. Obesity 14:309–318
6. **Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR** 1999 Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94:2467–2474
7. **Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ** 2005 Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41:1313–1321
8. **Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, Coussieu C, Basdevant A, Bar Hen A, Bedossa P, Guerre-Millo M, Clément K** 2006 Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. Diabetes 55:1554–1561
9. **Aron-Wisnewsky J, Tordjman J, Poitou C, Darakhshan F, Hugol D, Basdevant A, Aissat A, Guerre-Millo M, Clément K** 2009 Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. J Clin Endocrinol Metab 94:4619–4623
10. **Swarbrick MM, Stanhope KL, Austrheim-Smith IT, Van Loan MD, Ali MR, Wolfe BM, Havel PJ** 2008 Longitudinal changes in pancreatic and adipocyte hormones following Roux-en-Y gastric bypass surgery. Diabetologia 51:1901–1911
11. **Faraj M, Havel PJ, Phélis S, Blank D, Sniderman AD, Cianflone K** 2003 Plasma acylation-stimulating protein, adiponectin, leptin, and ghrelin before and after weight loss induced by gastric bypass surgery in morbidly obese subjects. J Clin Endocrinol Metab 88:1594–1602
12. **Weigert J, Neumeier M, Wanninger J, Filarsky M, Bauer S, Wiest R, Farkas S, Scherer MN, Schäffler A, Aslanidis C, Schölmerich J, Buechler C** 24 June 2009 Systemic chemerin is related to inflammation rather than obesity in type 2 diabetes. Clin Endocrinol (Oxf) doi: 10-1111/j.1365-2265.2009.03664.x
13. **Tan BK, Chen J, Farhatullah S, Adya R, Kaur J, Heutling D, Lewandowski KC, O'Hare JP, Lehnert H, Randeva HS** 2009 Insulin and metformin regulate circulating and adipose tissue chemerin. Diabetes 58:1971–1977
14. **Sell H, Laurencikiene J, Taube A, et al** 2009 Chemerin is a novel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. Diabetes 58:3731–3740
15. **Argentou M, Tiniakos DG, Karanikolas M, Melachrinou M, Makri MG, Kittas C, Kalfarentzos F** 2009 Adipokine serum levels are related to liver histology in severely obese patients undergoing bariatric surgery. Obes Surg 19:1313–1323
16. **García-Galiano D, Sánchez-Garrido MA, Espejo I, Montero JL, Costán G, Marchal T, Membrives A, Gallardo-Valverde JM, Muñoz-Castañeda JR, Arévalo E, De la Mata M, Muntané J** 2007 IL-6 and IGF-1 are independent prognostic factors of liver steatosis and nonalcoholic steatohepatitis in morbidly obese patients. Obes Surg 17:493–503
17. **Tordjman J, Poitou C, Hugol D, Bouillot JL, Basdevant A, Bedossa P, Guerre-Millo M, Clement K** 2009 Association between omental adipose tissue macrophages and liver histopathology in morbid obesity: influence of glycemic status. J Hepatol 51:354–362
18. **Takahashi M, Takahashi Y, Takahashi K, Zolotaryov FN, Hong KS, Kitazawa R, Iida K, Okimura Y, Kaji H, Kitazawa S, Kasuga M, Chihara K** 2008 Chemerin enhances insulin signaling and potentiates insulin-stimulated glucose uptake in 3T3-L1 adipocytes. FEBS Lett 582:573–578