Insulin regulation of MCP-1 in human adipose tissue of obese and lean women

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1University of Helsinki, Department of Medicine, Division of Diabetes, and 2Minerva Medical Research Institute, Helsinki, Finland; 3Atherosclerosis Research Unit, King Gustaf V Research Institute, Karolinska Institutet, Stockholm, Sweden; 4HUSLAB, Helsinki University Central Hospital, Helsinki, Finland

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Westerbacka J, Cornér A, Kolak M, Makkonen J, Turpeinen U, Hamsten A, Fisher RM, Yki-Järvinen H. Insulin regulation of MCP-1 in human adipose tissue of obese and lean women. Am J Physiol Endocrinol Metab 294: E841–E845, 2008. First published 12 February 2008; doi:10.1152/ajpendo.00653.2006.—CCL2 (MCP-1, monocyte chemoattractant protein 1) and CCL3 (MIP-1α, macrophage inflammatory protein 1α) are required for macrophage infiltration in adipose tissue. Insulin increases CCL2 expression in adipose tissue and in serum more in insulin-resistant obese than in insulin-sensitive lean mice, but whether this is true in humans is unknown. We compared basal expression and insulin regulation of CCL2 and CCL3 in adipose tissue and MCP-1 and MIP-1α in serum between insulin-resistant and insulin-sensitive human subjects. Subcutaneous adipose tissue biopsies and blood samples were obtained before and at the end of 6 h of in vivo euglycemic hyperinsulinemia (maintained by the insulin clamp technique) in 11 lean insulin-sensitive and 10 obese insulin-resistant women, and before and after a 6-h saline infusion in 8 women. Adipose tissue mRNA concentrations of monocyte/macrophage markers CD68, EMR1, ITGAM, ADAM8, chemokines CCL2 and CCL3, and housekeeping gene ribosomal protein large P0 (RPLP0) were measured by means of real-time PCR at baseline. In addition, mRNA concentrations of CCL2, CCL3, and RPLP0 were measured after insulin infusion. Levels of MCP-1 and MIP-1α were determined in serum, and protein concentration of MCP-1 was determined in adipose tissue at baseline and after insulin infusion. Basally, expression of the macrophage markers CD68 and EMR1 were increased in adipose tissue of insulin-resistant subjects. Insulin increased MCP-1 gene and protein expression significantly more in the insulin-resistant than in the insulin-sensitive subjects. Basally, expression of CCL2 and CCL3 and expression of macrophage markers CD68 and ITGAM were significantly correlated. In serum, MCP-1 decreased significantly in insulin-sensitive but not insulin-resistant subjects. MIP-1α was undetectable in serum. Insulin regulation of CCL2 differs between insulin-sensitive and -resistant subjects in a direction that could exacerbate adipose tissue inflammation.

MCP-1, CCL2 (monocyte chemoattractant protein 1), and CCL3 (MIP-1α, macrophage inflammatory protein 1α) are required for macrophage infiltration in adipose tissue. Insulin increases CCL2 expression in adipose tissue and in serum more in insulin-resistant obese than in insulin-sensitive lean mice, but whether this is true in humans is unknown. We compared basal expression and insulin regulation of CCL2 and CCL3 in adipose tissue and MCP-1 and MIP-1α in serum between insulin-resistant and insulin-sensitive human subjects. Subcutaneous adipose tissue biopsies and blood samples were obtained before and at the end of 6 h of in vivo euglycemic hyperinsulinemia (maintained by the insulin clamp technique) in 11 lean insulin-sensitive and 10 obese insulin-resistant women, and before and after a 6-h saline infusion in 8 women. Adipose tissue mRNA concentrations of monocyte/macrophage markers CD68, EMR1, ITGAM, ADAM8, chemokines CCL2 and CCL3, and housekeeping gene ribosomal protein large P0 (RPLP0) were measured by means of real-time PCR at baseline. In addition, mRNA concentrations of CCL2, CCL3, and RPLP0 were measured after insulin infusion. Levels of MCP-1 and MIP-1α were determined in serum, and protein concentration of MCP-1 was determined in adipose tissue at baseline and after insulin infusion. Basally, expression of the macrophage markers CD68 and EMR1 were increased in adipose tissue of insulin-resistant subjects. Insulin increased MCP-1 gene and protein expression significantly more in the insulin-resistant than in the insulin-sensitive subjects. Basally, expression of CCL2 and CCL3 and expression of macrophage markers CD68 and ITGAM were significantly correlated. In serum, MCP-1 decreased significantly in insulin-sensitive but not insulin-resistant subjects. MIP-1α was undetectable in serum. Insulin regulation of CCL2 differs between insulin-sensitive and -resistant subjects in a direction that could exacerbate adipose tissue inflammation.

MCP-1 plays a key role in recruitment of monocytes but not neutrophils or eosinophils to sites of injury (6, 26). It also induces insulin resistance in adipocytes via downregulation of genes such as SLC2A4 (the gene encoding GLUT-4), lipoprotein lipase, and peroxisome proliferator-activated receptor-γ (16). Recently, it was demonstrated that deletion of the CCR2 receptor for monocyte chemoattractant protein (MCP)-1 in obese mice strains matched for adiposity reduced macrophage content and the inflammatory profile of adipose tissue, increased adiponectin expression, ameliorated hepatic steatosis, and improved systemic glucose homeostasis and insulin sensitivity (21). In mice with established obesity, short-term treatment with a pharmacological antagonist of MCP-1 lowered macrophage content of adipose tissue and improved insulin sensitivity without significantly altering body mass or improving hepatic steatosis (21).

In murine adipocytes in vitro and ob/ob mice in vivo, insulin increases expression and secretion of MCP-1 (16). In the latter in vivo experiment, the response to insulin was exaggerated in obese insulin-resistant compared with lean mice (16). Thus, as previously described for plasminogen activator inhibitor (PAI)-1 (15) and sterol regulatory element binding protein (SREBP)-1c (17) in mice, MCP-1 may retain its sensitivity to insulin or even hyperrespond to insulin in insulin-resistant states. Hyperinsulinemia might under such conditions accelerate monocyte recruitment and worsen insulin resistance (16). These data may or may not be relevant to humans, since insulin has been reported to decrease serum MCP-1 levels in humans (5), and since catheterization studies of human subcutaneous tissues have suggested that MCP-1 is not released systematically (4). There are no studies comparing the response of CCL2 expression or of its levels in serum to insulin between insulin-sensitive and insulin-resistant human subjects.

In the present study, we examined how acute in vivo hyperinsulinemia in human insulin-resistant compared with insulin-sensitive subjects regulates expression of the chemokines CCL2 and CCL3 and MCP-1 protein concentration in adipose tissue. We also determined whether expression of multiple macrophage markers basally is correlated with chemokine expression. The latter genes included CD68 and EMR1 (epidermal growth factor module-containing mucin-like hormone receptor 1), and ADAM8 (a disintegrin and metalloproteinase domain 8), which are macrophage-specific markers, and ITGAM (integrin, alpha M), which is an integrin found in monocytes, macrophages, neutrophils, and NK cells.

MATERIALS AND METHODS

Subjects and study designs. A total of 21 nondiabetic Caucasian women were recruited based on the following inclusion criteria: 1) age 18–60 years; 2) no known acute or chronic disease other than obesity based on history and physical examination and standard content and the inflammatory profile of adipose tissue, increased adiponectin expression, ameliorated hepatic steatosis, and improved systemic glucose homeostasis and insulin sensitivity (21). In mice with established obesity, short-term treatment with a pharmacological antagonist of MCP-1 lowered macrophage content of adipose tissue and improved insulin sensitivity without significantly altering body mass or improving hepatic steatosis (21).

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laboratory tests (blood counts, serum creatinine, thyroid stimulating hormone, electrolyte concentrations, and electrocardiogram); and 3) body mass index (BMI) < 40 kg/m2. Other exclusion criteria included treatment with drugs that may alter glucose tolerance or pregnancy. In each subject, whole-body insulin sensitivity was measured according to the euglycemic insulin clamp technique (insulin infusion rate 1 mU·kg⁻¹·min⁻¹ for 6 h), and needle biopsies of adipose tissue were taken before and after 6 h of hyperinsulinemia. The women were divided into lean insulin-sensitive (n = 11, age 32 ± 3 yrs, body wt 69 ± 4 kg, BMI 24.7 ± 1.1 kg/m2) and obese insulin-resistant (n = 10, age 40 ± 3 yrs, body wt 90 ± 4 kg, BMI 32.7 ± 1.8 kg/m2) based on their median rate of whole-body insulin sensitivity measured by the euglycemic insulin clamp technique (23). There were two postmenopausal women in both groups. In 8 women (age 36 ± 4 yrs, BMI 28.2 ± 2.9 kg/m2), CCL2 and CCL3 expressions were measured in adipose tissue before and at the end of a 6-h saline infusion.

The nature and potential risks of the study were explained to all subjects prior to obtaining their written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki. The protocol was approved by the ethics committee of the Helsinki University Central Hospital.

Adipose tissue biopsy and total RNA cDNA preparation. Needle aspiration biopsies of abdominal subcutaneous fat were taken under local intracutaneous anesthesia at baseline and after 6 h of hyperinsulinemia from the left and right lower abdominal region (25). The samples were immediately frozen and stored in liquid nitrogen until analysis. Frozen tissue samples (50–150 mg) were homogenized in 2 ml RNA STAT-60 (Tel-Test, Friendswood, TX), and total RNA was isolated as previously described (19). RNA was stored at −80°C until quantification of target mRNAs. A total of 0.1 μg RNA was transcribed into cDNA via Moloney murine leukemia virus reverse transcriptase (Life Technologies, Paisley, UK) and oligo (dT)12–18 primers (19). Part of the fresh sample was used to isolate adipocytes through incubation with collagenase to measure adipocyte size with microscope (7).

Gene expression analyses. mRNA expression of CD68, EMR1, ITGAM, ADAM8, CCL2, CCL3, and ribosomal protein large P0 (RPLP0) was quantified by real-time PCR by means of the ABI 7000 Sequence Detection System instrument and software (Applied Biosystems). cDNA synthesized from 15 ng of total RNA was mixed with TaqMan Universal PCR Master Mix (Applied Biosystems) and a gene-specific primer and probe mixture (predeveloped TaqMan Gene Expression Assays, Applied Biosystems) in a final volume of 15 μl. The assays used were: CD68, Hs00154355_m1; EMR1, Hs00173562_m1; ITGAM, Hs00355885_m1; ADAM8, Hs00174246_m1; CCL2, Hs00234140_m1; CCL3, Hs00234142_m1; and RPLP0, Hs99999902_m1. All samples were run in duplicate. Relative expression levels were determined by means of a 5-point serially diluted standard curve, generated from cDNA from human adipose tissue. Expression levels were expressed in arbitrary units and normalized relative to the housekeeping gene RPLP0 to compensate for differences in cDNA loading.

Measurement of MCP-1 protein expression in adipose tissue. A frozen sample (5 from the insulin-sensitive and 7 from the insulin-resistant group) of human subcutaneous adipose tissue (100–250 mg) was homogenized in lysis buffer. The homogenate was centrifuged for 30 min (+4°C, 14,000 rpm), and the supernatant was stored at −80°C until measurement of the MCP-1 concentration with the Human CCL2/MCP-1 Immunoassay kit (Quantikine; R&D Systems, Minneapolis, MN) and the Bio Assay Reader HTS 7000 Plus (Perkin Elmer, Norwalk, CT). Total protein was measured with the BC Assay-protein quantitation kit (Uptima Interchim, Montlucan, France).

Other measurements. Blood samples were taken after an overnight fast for measurement of plasma glucose, serum insulin, C-peptide, serum triglycerides, and total and HDL cholesterol concentrations, as described (23). Serum MCP-1 and macrophage inflammatory protein (MIP)-1α concentrations were measured with ELISA kits (Quantikine, R&D Systems, Minneapolis, MN, USA).

Statistical analyses. All parameters were analyzed via nonparametric methods. Insulin-sensitive and insulin-resistant groups were compared via the Mann-Whitney test. Effects of insulin were analyzed via Friedman’s test followed by Dunn’s post hoc test to compare single measurements. Correlations were calculated via Spearman’s rank correlation coefficient. A P value of less than 0.05 was considered statistically significant. The calculations were performed with SPSS 11.0 for Windows (SPSS, Chicago, IL). All data are shown as mean ± standard error of mean.
RESULTS

The insulin-resistant group was more obese than the insulin-sensitive group (BMI 32.7 ± 1.8 vs. 24.7 ± 1.1, P < 0.001), and had higher insulin concentrations (10 ± 1 vs. 3 ± 1 mU/L, P < 0.001). During the insulin infusion, serum insulin concentrations were similar in insulin-sensitive and insulin-resistant groups (69 ± 4 vs. 76 ± 4 mU/L, insulin-sensitive vs. insulin-resistant, NS). Whole-body insulin sensitivity was 95% higher in the insulin-sensitive than in the insulin-resistant group (8.7 ± 0.4 vs. 4.2 ± 0.3 mg·kg⁻¹·min⁻¹, P < 0.0001). Other characteristics have been described in (23).

Gene expressions of CD68, EMR1, ITGAM, ADAM8, CCL2 (MCP-1), and CCL3 (MIP-1α), and MCP-1 protein concentration in adipose tissue. Before start of the insulin infusion, the mRNA concentrations of ITGAM, ADAM8, CCL2, and CCL3 were comparable between the insulin-sensitive and insulin-resistant groups, but the mRNA concentrations of CD68 and EMR1 were significantly higher in the insulin-resistant than in the insulin-sensitive group (Fig. 1). At baseline, CCL2 and CCL3 expressions correlated closely with those of CD68 (r = 0.71, P < 0.001 and r = 0.57, P < 0.001 for CCL2 and CCL3, respectively) and ITGAM (r = 0.64, P < 0.01 and r = 0.78, P < 0.001, respectively), and CCL3 correlated with EMR1 (r = 0.55, P < 0.01). CCL2 expression increased significantly more by insulin in the insulin-resistant than in the insulin-sensitive group (Fig. 2). The % increase in CCL2 gene expression was 60 ± 22% in insulin-sensitive and 169 ± 59% in insulin-resistant subjects (P = 0.08). Similarly, the protein concentration of MCP-1 in adipose tissue increased significantly by insulin in the insulin-resistant group but remained unchanged in the insulin-sensitive group (Fig. 3). CCL2 expression and MCP-1 protein concentration in adipose tissue were significantly correlated (r = 0.53, P = 0.008). In the 8 subjects who received a 6-h saline infusion, expressions of CCL2 and CCL3 remained unchanged (data not shown). Adipocyte size correlated significantly with the mRNA concentration of CD68 (r = 0.63, P = 0.024).

Whole-body insulin sensitivity was inversely correlated with basal expression of macrophage markers as follows: CD68 (Spearman’s r = −0.58, P < 0.01), EMR1 (r = −0.62, P < 0.01), ITGAM (r = −0.50, P < 0.05), ADAM8 (r = −0.42, P = 0.058, NS). After adjustment for BMI and age, these correlations were as follows: CD68 (r = −0.44, P < 0.05), EMR1 (r = −0.28, NS), ITGAM (r = −0.67, P < 0.001), ADAM8 (r = −0.21, NS). Whole-body insulin sensitivity correlated with CCL2 (r = −0.60, P < 0.01) and CCL3 (r = −0.73, P < 0.001) expression in adipose tissue at 6 h.

Serum MCP-1 and MIP-1α concentration. At baseline, serum MCP-1 concentrations were comparable in the insulin-sensitive and insulin-resistant groups (291 ± 19 vs. 343 ± 34 pg/ml, insulin-sensitive vs. insulin-resistant, NS). Insulin decreased serum MCP-1 concentration significantly in the insulin-sensitive group but not in the insulin-resistant group (Fig. 3).
At 6 h, serum MCP-1 concentration was significantly lower in the insulin-sensitive than in the insulin-resistant group (216 ± 14 vs. 313 ± 26 pg/ml, \( P < 0.01 \) insulin-sensitive vs. insulin-resistant). Serum MIP-1\( \alpha \) was under the detection limit of the assay (46.9 pg/ml) in all subjects.

**DISCUSSION**

The present data are the first to compare responses of the chemokines MCP-1 and MIP-1\( \alpha \) to insulin in vivo in adipose tissue between insulin-resistant obese and insulin-sensitive lean women. In vivo euglycemic hyperinsulinemia increased gene and protein expression of MCP-1 (CCL2) more in adipose tissue and decreased MCP-1 less in serum in insulin-resistant than in insulin-sensitive subjects. We also found that whole-body insulin resistance was closely correlated with increased expression of the monocyte/macrophage markers. An increase in basal MCP-1 gene and protein expression has previously been documented in human adipocyte cultures from obese compared with lean subjects (1, 4), and in adipose tissue of ob/ob mice (16). Regarding expression of CCL2 in adipose tissue, we have previously shown that CCL2 correlates significantly with the concentration of MCP-1 protein in adipose tissue (9).

Classically, insulin resistance has been defined as a defective response of an insulin-sensitive gene, protein, or pathway to insulin. In recent years, data from animal studies have suggested that this concept needs to be expanded. Insulin not only regulates molecules and pathways that normally enhance insulin sensitivity, but also molecules that normally confer insulin resistance. Examples of such molecules include TNF-\( \alpha \) (8, 23), 11\( \beta \)-HSD-1 (10, 20), and PAI-1 (15) and SREBP-1c (17), which continue to respond to insulin in insulin-resistant states. In the present study, we found CCL2 gene expression in adipose tissue to hyperrespond to insulin in the insulin-resistant subjects (Fig. 2). This finding resembles the transient hyperresponse to insulin (peaking at 1 h in db/db and at 3 h in wild-type mice) described in 3T3-L1 adipocytes made insulin resistant by TNF-\( \alpha \) treatment in vitro (16). The insulin-resistant 3T3-L1 adipocytes also overproduced MCP-1 protein. In vivo, insulin increased MCP-1 mRNA concentrations in adipose tissue of ob/ob mice more than in wild-type mice. There was also a transient increase after 1 h of insulin injection in MCP-1 protein in plasma in the ob/ob mice, which was lacking from the wild-type insulin sensitive mice, in which serum MCP-1 levels remained unchanged for 6 h (16). The present data showing an exaggerated increase in CCL2 gene and protein expression in adipose tissue in insulin-resistant obese compared with insulin-sensitive lean subjects are consistent with data in mice. However, in serum, MCP-1 decreased significantly in the control group, consistent with another study also showing a decrease in serum MCP-1 by insulin but not by saline in vivo (5). We did not detect any change in serum MCP-1 in the insulin-resistant group when the level was measured at 0 and 6 h. The data suggest that adipose tissue is not the main determinant of circulating MCP-1 levels in humans. Consistent with this, a catheterization study in humans found no release of MCP-1 from subcutaneous adipose tissue to the circulation (4). Serum MIP-1\( \alpha \) levels were undetectable in all subjects. In a study using the same assay, only 15% of the type 2 diabetic patients had detectable concentration of MIP-1\( \alpha \) in serum (12).

We also confirm increased basal expression of the macrophage markers CD68 in human adipose tissue in insulin-resistant subjects (1, 22) and report an increase in EMR1. CD68 (3), ADAM-8 (24), and EMR1 (11) are macrophage-specific markers, and ITGAM is a member of an integrin family mediating leukocyte adhesion and migration processes (18). This increase in macrophage gene expression is likely to reflect an increase in macrophage number based on previous data (22), including our own (9). Obesity per se may be a confounding factor when interpreting the data regarding associations to insulin sensitivity. However, in the present study there were also two overweight (BMI 27–30 kg/m\(^2\)) subjects and one obese subject in the insulin-sensitive group. None of the insulin-resistant subjects had a BMI < 27 kg/m\(^2\).

The present study did not address the molecular mechanisms explaining how a gene can hyperrespond to insulin despite whole-body insulin resistance, which reflect mostly insulin resistance at the level of skeletal muscle (13). It is of interest in this respect that insulin inhibition of hormone-sensitive lipase in macrophages, unlike in adipocytes, does not involve activation of the PI 3-kinase pathway (14). In ob/ob mice, macrophages are characterized by reduced insulin receptor expression and signaling, which leads to upregulation of, e.g., the proatherogenic CD36, a scavenger receptor-accumulating lipid. It has recently been demonstrated that macrophages are localized to dead adipocytes (2) and that the rate of adipocyte death is dramatically increased in obesity. Consistent with these data, macrophage marker CD68 expression was significantly correlated with adipocyte size in the present study. Free lipid droplets of dead adipocytes are engulfed by macrophages, which become multinucleated giant cells (2). Against this background, the ability of hyperinsulinemia to promote inflammation in adipose tissue might be viewed as a protective mechanism which help to facilitate removal of dead adipocytes.

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**REFERENCES**


