

Monocyte Chemoattractant Protein-1 in Obesity and Type 2 Diabetes. Insulin Sensitivity Study

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Abstract

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Objective: Our goal was to test any association between human plasma circulating levels of monocyte chemoattractant protein-1 (cMCP-1) and insulin resistance and to compare monocyte chemoattractant protein-1 (MCP-1) adipose tissue gene expression and cMCP-1 in relation with inflammatory markers.

Research Methods and Procedures: cMCP-1 was measured in $n = 116$ consecutive control male subjects to whom an insulin sensitivity (S_i) test was performed. Circulating levels of soluble CD14, soluble tumor necrosis factor receptor type 2 (sTNFR2), soluble interleukin-6 (sIL-6), and adiponectin also were measured. Subcutaneous adipose tissue samples were obtained from $n = 107$ non-diabetic and type 2 diabetic subjects with different degrees of obesity. Real-time polymerase chain reaction was used to measure gene expression of *MCP-1*, *CD68*, *tumor necrosis factor- α* (*TNF- α*), and its receptor *TNFR2*.

Results: In the S_i study, no independent effect of cMCP-1 levels on insulin sensitivity was observed. In the expression study, in non-diabetic subjects, MCP-1 mRNA had a positive correlation with BMI ($r = 0.407$, $p = 0.003$), TNF- α mRNA ($r = 0.419$, $p = 0.002$), and TNFR2 mRNA ($r = 0.410$, $p = 0.003$). In these subjects, cMCP-1 was found to correlate with waist-to-hip ratio ($r = 0.322$, $p = 0.048$). In patients with type 2 diabetes, MCP-1 mRNA was up-regulated compared with non-diabetic subjects. TNF- α mRNA was found to independently contribute to MCP-1 mRNA expression. In this group, CD68 mRNA was found to correlate with BMI ($r = 0.455$, $p = 0.001$).

Discussion: cMCP-1 is not associated with insulin sensitivity in apparently healthy men. TNF- α is the inflammatory cytokine associated with MCP-1 expression in subcutaneous adipose tissue.

Key words: monocyte chemoattractant protein-1, adipose tissue, insulin sensitivity, type 2 diabetes

Introduction

Monocyte chemoattractant protein-1 (MCP-1)¹ is a well-characterized CC chemokine with a potent agonist effect for monocytes, memory T cells, and basophiles (1). MCP-1 has been implicated as a key player in the recruitment of monocytes to atheroma (2,3), and the overexpression of MCP-1 in specific tissues causes a localized infiltration of monocyte/macrophages. It is produced by a variety of cells, mainly by macrophages and endothelial cells (4).

Obesity is frequently accompanied by a strong association with cardiovascular disease, and it is related to many factors that are constituents of the metabolic syndrome. Increasing evidence suggests that features of the metabolic

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¹ Nonstandard abbreviations: MCP-1, monocyte chemoattractant protein-1; IL, interleukin; TNF, tumor necrosis factor; cMCP-1, circulating monocyte chemoattractant protein-1; S_i , insulin sensitivity; WHR, waist-to-hip ratio; TNFR2, TNF receptor type 2; RT, reverse transcriptase.

syndrome, including obesity and type 2 diabetes, have a common inflammatory basis (5). A considerable body of evidence supports the notion that various mediators such as cytokines and chemokines are involved in the process of atherosclerotic lesions (1,3).

Adipose tissue is now considered a metabolically active organ with the capacity to synthesize and secrete various chemokines and pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) (6). During the development of obesity and type 2 diabetes, this tissue dramatically increases its metabolic activity, suggesting that some of the adipocyte-derived factors could lead to the association of insulin resistance and increased risk for cardiovascular (accelerated atherothrombotic) disease. Several works have shown that, in metabolic situations of insulin resistance accompanied by circulating hyperinsulinemia, several insulin-responding genes may remain sensitive to insulin, inducing an increase in terms of gene expression in target tissues (7,8). In this sense, MCP-1 mRNA is elevated in the adipose tissue of genetically obese mice (*ob/ob* and diet-induced obese mice), remaining responsive to exogenous insulin both in mice and in insulin-resistant 3T3L-1 adipocytes (9).

It has been shown that MCP-1 may be released from human preadipocytes as well as from isolated mature adipocytes (10), with an increased secretion and expression in human obesity (11,12). MCP-1 protein and gene expression was found to be more expressed in visceral than in subcutaneous adipose tissue (11). However, in humans, the correlation between cMCP-1 and its local adipose tissue secretion has shown discordant results (12,13).

Based on the hypothesis that this chemokine could be an important link between adipose tissue inflammation and insulin resistance, we studied the relationship between circulating MCP-1 (cMCP-1) plasma levels and insulin resistance in a large group of control white men, to whom an insulin sensitivity (S_i) test was performed. Additionally, we aimed to study MCP-1 adipose tissue gene expression in relationship with cMCP-1 plasma protein levels in a representative group of non-diabetic and type 2 diabetic subjects with different degrees of obesity. In this population, the association between *MCP-1* gene expression and the expression of the inflammatory markers TNF- α and CD68 also was studied.

Research Methods and Procedures

Insulin Sensitivity Study (Control Male Cohort)

One hundred sixteen consecutive, unselected (except for inclusion criteria, see below) white men (mean, 50.22 \pm 11.46 years), participants in an ongoing epidemiological study of risk factors for cardiovascular disease in Northern Spain, were included in the study (Table 1). Subjects were randomly localized from a census, and they were invited to

participate. The participation rate was 71%. Non-diabetic subjects had fasting plasma glucose <7.0 mM and 2-hour post-load plasma glucose <11.1 mM after a 75-gram oral glucose tolerance test according to the American Diabetes Association criteria. With these criteria, there were 32% of subjects with impaired fasting glucose or impaired glucose tolerance and 68% with a normal oral glucose tolerance test. None of the subjects was taking any medication or had any evidence of metabolic disease other than obesity. (Patients taking thiazolidinediones were excluded from the study.) Inclusion criteria were 1) BMI <40 kg/m², 2) absence of systemic disease, and 3) absence of infection within the previous month.

Insulin sensitivity was measured using the frequently sampled intravenous glucose tolerance test with minimal model analysis, as previously described (14). In brief, intravenous glucose (0.3 g/kg) was administered at time 0, and insulin (0.03 U/kg) at time +20 minutes. The time-courses of serum glucose and insulin during 3 hours were analyzed using the Minimal Model Program to calculate the S_i index.

Adipose Tissue Gene Expression Study

Lean and Non-severely Obese Population. A group of 29 lean subjects (15 with type 2 diabetes) and 52 non-severely obese subjects (26 with type 2 diabetes) were recruited at the Endocrinology Service of the Hospital Universitari Joan XXIII and at the Surgical Service of Hospital Sant Pau i Santa Tecla (Tarragona, Spain). All subjects were of white origin and reported that their body weight had been stable for at least 3 months before the study. They had no systemic disease other than obesity or type 2 diabetes, and all were free of any infections in the previous month before the study. Liver and renal diseases were specifically excluded by biochemical work-up. Patients were classified as having type 2 diabetes according to American Diabetes Association criteria, with a stable metabolic control in the previous 6 months, as defined by stable hemoglobin A_{1c} values. Exclusion criteria included the following: 1) clinically significant hepatic, neurologic, or other major systemic disease, including malignancy; 2) history or current clinical evidence of hemochromatosis; 3) history of drug or alcohol abuse, defined as >80 grams of alcohol intake per day in men and >40 grams of alcohol intake per day in women, or serum transaminase activity more than twice the upper limit of normal; 4) an elevated serum creatinine concentration; 5) acute major cardiovascular event in the previous 6 months; and 6) acute illnesses and current evidence of acute or chronic inflammatory or infective diseases. Pharmacologic treatment of the patients with type 2 diabetes was as follows: insulin, 25%; oral hypoglycemic agents, 66.6% (sulfonylurea: 80%; metformin: 20%); statins, 58.3%; fibrates, 10.6%; blood pressure-lowering agents: 53.8%.

Table 1. Clinical and analytical characteristics of the men ($n = 116$) included in the insulin sensitivity study

Age (years)	50.22 ± 11.46
BMI (kg/m ²)	27.39 ± 3.42
WHR	0.94 ± 0.06
Diastolic blood pressure (mm Hg)	79.44 ± 9.53
Systolic blood pressure (mm Hg)	127.30 ± 13.44
Hemoglobin A _{1c} (%)	4.77 ± 0.39
Fasting glucose (mM)	5.32 ± 0.50
Fasting insulin (mU/L)	9.82 ± 5.6
S _i (μmol/kg/min)	2.77 ± 1.9
Total cholesterol (mM)	5.63 ± 1.19
Triglycerides (mM)	1.03 (1.54)
High-density lipoprotein-cholesterol (mM)	1.22 ± 0.34
Low-density lipoprotein-cholesterol (mM)	3.83 ± 1.04
sTNFR2 (ng/mL)	4 (4.7)
Adiponectin (μg/mL)	6.34 (8.8)
sIL-6 (ng/L)	1 (1.4)
Soluble CD14 (ng/mL)	4.3 (5.32)
cMCP-1 (pg/mL)	367.1 (507.72)

WHR, waist-to-hip ratio; S_i, insulin sensitivity; sTNFR2, soluble tumor necrosis factor receptor type 2; sIL, soluble interleukin; cMCP-1, circulating monocyte chemoattractant protein-1. Normally distributed data are presented as mean ± standard deviation. Non-normally distributed data are presented as the median (75th percentile).

Severely Obese Subjects. Twenty-six severely obese subjects (15 with type 2 diabetes) of white origin were included in the study. All patients were recruited in the Endocrinology Service of the Hospital Universitari de Bellvitge (Barcelona, Spain) where they have been scheduled for gastric bypass surgery. Preoperative anthropometric measurements were made, and blood samples were collected before surgical procedure. Patients were excluded if they had an acute major cardiovascular event in the previous 6 months, an acute illness, or current evidence of acute or chronic inflammatory or infectious disease.

Anthropometric Measurements

Height was measured to the nearest 0.5 cm and body weight to the nearest 0.1 kg. BMI was calculated as weight (in kilograms) divided by height (in square meters). Waist circumference was measured midway between the lower rib margin and the iliac crest. Hip circumference was determined as the widest circumference measured over the greater trochanter. Waist-to-hip ratio (WHR) was calcu-

lated. Blood pressure was measured in the supine position on the right arm after a 10-minute rest; a standard sphygmomanometer of appropriate cuff size was used, and the first and fifth phases were recorded.

Analytical Methods

Blood samples were drawn from each subject before breakfast or before surgical procedure between 8:00 AM and 9:00 AM, after an overnight bed rest. Plasma and serum samples were stored at -80 °C until analytical measurements were performed, except for glucose, which was immediately determined after blood was drawn.

Plasma glucose was measured using the glucose analyzer YSI 2300 STAT Plus (YSI, Yellow Springs, OH). Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. High-density lipoprotein-cholesterol was quantified after precipitation with polyethylene glycol at room temperature. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase. Hemoglobin A_{1c} was measured by the high-performance liquid chromatography method (Bio-Rad, Munchen, Germany) with a recommended normal range of the assay being 4.1% to 6.5%.

Soluble CD14 was determined by solid phase enzyme immunoassay (Biosource Europe, Fleunes, Belgium). The minimum detectable concentration was estimated to be 1 ng/mL. The intra- and inter-assay coefficients of variation were <5.2% and 7.8%, respectively. No plasma proteins showed any cross-reactivity in this assay.

Plasma soluble MCP-1 levels (cMCP-1) were measured by an (h)MCP-1 Biotrak enzyme-linked immunosorbent assay kit (Amersham Biosciences, Buckinghamshire, UK); the sensitivity of the assay was <10 pg/mL, and the coefficient of variation was <10%.

Plasma soluble TNF receptor type 2 (TNFR2) was determined by a solid phase enzyme-immunoassay kit (BioSource). The minimum detectable concentration was 0.1 ng/mL. The intra- and inter-assay coefficients of variation were <6.5% and <9%, respectively. TNF-α does not interfere with the assay.

Plasma IL-6 was determined by an ultrasensitive solid phase enzyme-immunoassay (BioSource). Plasma high-sensitive C reactive protein was measured by a highly sensitive immunonephelometry kit (Dade Behring, Marburg, Germany). Plasma adiponectin was measured by radioimmunoassay (Linco Research, St. Charles, MO). The intra- and inter-assay coefficients of variation were <5%.

Adipose Tissue Samples

Adipose tissue samples from the 107 non-diabetic and type 2 diabetic subjects were obtained from subcutaneous abdominal depots during abdominal elective surgical procedures (gastric bypass operation, cholecystectomy, or sur-

gery of abdominal hernia). All patients had fasted overnight, and at the beginning of surgery, 2 to 4 grams of subcutaneous fat tissue was removed by scalpel from each proband and immediately introduced in RNeasy Lysis Buffer (Qiagen, Germantown, MD) for RNA extraction. The hospital's committee on ethics approved the study, and signed informed consent was obtained from each participant.

Total RNA Extraction and Reverse Transcription

Total RNA from 200 to 500 mg of adipose tissue samples was extracted using the RNeasy Lipid Midi Kit (Qiagen Science, Germantown, MD) following the manufacturer's instructions. RNA was treated with 55 U of RNase-free DNase (Qiagen) before column elution to avoid contamination with genomic DNA. The RNA integrity was electrophoretically verified by ethidium bromide staining and its purity by OD_{260}/OD_{280} nm absorption ratio.

One microgram of RNA was reverse transcribed (RT) to cDNA using a reverse transcription system (Promega, Madison, WI); 20 μ L of RT mixture contained: 1 \times RT-buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% TritonX-100), 1 mM of each deoxyribonucleotide triphosphate, 1 U/ μ L recombinant RNasin ribonuclease inhibitor, 15 U of avian myeloblastosis virus RT, and 0.5 μ g of random primers. The cDNA reaction was incubated for 10 minutes at 25 °C followed by 60 minutes at 42 °C and heated 5 minutes at 95 °C.

Real-time Polymerase Chain Reaction

Real-time quantitative polymerase chain reaction analyses were performed with 2 μ L of cDNA on a LightCycler Instrument (Roche Diagnostics, Basel, Switzerland), using the SYBR green fluorescence method. The following primers were used: *MCP-1*, 5'tctgtgcctgctcatag3' and 5'cagatctcttgccacaat3'; *TNF- α* , 5'gagcactgaaagcatgatcc3' and 5'gctggttatctctcagctcca3'; *TNFR2*, 5'ggaactcaagcctgcactc3' and 5'ggatgaagtcgtgttgaga3'; and *CD68*, 5'atgaggctgctgtctt3' and 5'gtggtttgtgctcttgg3'. The housekeeping genes used to normalize gene expression were *β -actin*, 5'ggactcagcaagagatgg3' and 5'ctgtacgccaacacagtct3' and *cyclophilin A*, 5'caaatgctggaccaacac3' and 5'gcctccaatattcatgctctt3'. The reactions were performed in a volume of 20 μ L of a mixture containing 0.5 μ M of each oligonucleotide primer (except for *β -actin*, which was 0.2 μ M) and 2 μ L of DNA LC-FastStart DNA Master SYBR green I (Roche) containing Taq DNA polymerase, reaction buffer, deoxyribonucleotide triphosphate mix, and the double-stranded DNA-specific fluorescent dye SYBR green I. The final concentration of $MgCl_2$ was adjusted for each primer gene. LightCycler mastermix (18 μ L) was filled in the LightCycler glass capillaries, and 2 μ L cDNA was added as a polymerase chain reaction template. The following LightCycler protocol was used: denaturation program at 95 °C for 10 minutes, 45 cycles of denaturation at 95 °C for 10 minutes, annealing for 5 seconds at 60 °C for *TNF- α* and

cyclophilin A, 62 °C for *MCP-1*, 65 °C for *TNFR2*, 64 °C for *CD68*, and 66 °C *β -actin*, and 10 minutes of elongation at 72 °C.

Each amplified product was confirmed by agarose gel analysis and by melting curve analysis according to the manufacturer's manual.

Result Analysis and Gene Quantification

For each sample, the derived concentration was calculated from an external standard curve, created with serial dilutions of a cloned polymerase chain reaction fragment from the respective gene, using LightCycler Software v.3.5 (Roche Diagnostics).

Adipose tissue expression levels of each gene were normalized by *β -actin*. To determine whether *β -actin* was a good housekeeping gene for adipose tissue, the *cyclophilin A* gene was also evaluated in all of the study samples. Statistical analysis showed no differences for either gene between groups (data not shown).

Statistical Analysis

Statistical analysis was performed using the SPSS/PC+ statistical package (v. 11.5 for Windows; SPSS, Chicago, IL). Data are expressed as mean \pm standard deviation, but for non-normally distributed data, results are expressed as the median (75th percentile).

Differences in concentrations between groups were compared using a Student's *t* test or by ANOVA with a post hoc Bonferroni correction. Variables that did not have a Gaussian distribution were logarithmically transformed to perform statistical analysis. Relationships between variables were tested using Pearson's test and stepwise multiple linear regression analysis. Statistical significance occurred if a computed two-tailed probability value was <0.05 .

For the S_i study, the sample size was calculated to achieve a correlation of 0.25 or greater with a significance level of 95% and a statistical power of 80%. This will explain a variability of S_i of 6% or greater with respect to cMCP-1, which is the minimal percentage clinically acceptable. With these assumptions, the sample to be included resulted in 116 subjects.

Results

Insulin Sensitivity Study (Control Male Cohort)

The main anthropometric and analytical characteristics of the men included in the S_i study are shown in Table 1. In the simple correlation analysis, no significant associations between cMCP-1 protein levels and S_i or with other anthropometric and analytical measured variables were found in this population. S_i index was negatively correlated with age, BMI, and sIL-6 ($r = -0.2$, $p = 0.027$; $r = -0.57$, $p < 0.0001$; and $r = -0.23$, $p = 0.029$; respectively) and

Table 2. Characteristics of the non-diabetic and type 2 diabetic subjects

	Lean BMI ≤ 25 kg/m ²	Non-severely obese 25 < BMI ≤ 40 kg/m ²	Severely obese BMI > 40 kg/m ²
Non-diabetic subjects	(n = 14)	(n = 26)	(n = 11)
Age (years)	49.3 ± 18.04	57.85 ± 11.64	41.20 ± 11.89*
Sex (men/women)	9/5	14/12	1/10
BMI (kg/m ²)	22.91 ± 1.41	31.19 ± 4.24	51.48 ± 9.8†
WHR	0.89 ± 0.09	0.95 ± 0.06	0.97 ± 0.02
Fasting glucose (mM)	5.40 ± 0.47	5.61 ± 0.78	5.56 ± 0.70
cMCP-1 (pg/mL)	485.60 (545.15)	413.69 (556.05)	367.25 (448.4)
Human serum C reactive protein (mg/mL)	1.13 (4.68)	1.06 (7.09)	25.4 (26.6)
sTNFR2 (ng/mL)	6.11 (8.01)	4.37 (5.78)	6.74 (8.08)
Type 2 diabetic patients	(n = 15)	(n = 26)	(n = 15)
Age (years)	67.67 ± 12	66.27 ± 8.56	50 ± 10.18†
Sex (men/women)	13/2	14/12	0/15
BMI (kg/m ²)	23.14 ± 1.53	30.19 ± 3.56	49.60 ± 5.39†
WHR	0.94 ± 0.07	0.95 ± 0.06	0.92 ± 0.02
Fasting glucose (mM)	9.35 ± 2.73	9.20 ± 2.55	8.98 ± 3.32
Hemoglobin A _{1c} (%)	7.76 ± 1.23	6.64 ± 0.97	8.2 ± 1.27
cMCP-1 (pg/mL)	297.93 (392.85)	326.66 (406.89)	320 (521.65)
Human serum C reactive protein (mg/mL)	3.29 (5.75)	2.24 (7.14)	6.6 (17.7)
sTNFR2 (ng/mL)	5.98 (9.47)	2.24 (7.14)	6.91 (8.83)

WHR, waist-to-hip ratio; cMCP-1, circulating monocyte chemoattractant protein-1; sTNFR2, soluble tumor necrosis factor receptor type 2. Normally distributed data are presented as mean ± standard deviation, except for sex. Non-normally distributed data are presented as the median (75th percentile).

All comparisons were made with one-way ANOVA test with Bonferroni post hoc multiple comparisons.

* $p < 0.006$ compared with lean and non-severely obese.

† $p < 0.0001$ compared with lean and non-severely obese.

positively associated with adiponectin circulating levels ($r = 0.27, p = 0.013$), but not with cMCP-1 ($r = 0.16, p = 0.08$).

We constructed a multivariate regression analysis with S_i as the dependent variable, and BMI, age, sIL-6, cMCP-1, and adiponectin as independent variables. Only BMI and adiponectin appeared as independent determinants of S_i ($B = -0.05, p < 0.0001$ and $B = 0.24, p = 0.008$, respectively). No independent effect of circulating MCP-1 levels on S_i was detected in this model.

Hemoglobin A_{1c} had a weak positive correlation with cMCP-1 levels ($r = 0.19, p = 0.04$), and this association persisted after adjusting for age and BMI ($B = 0.09, p = 0.04$).

To know the factors that can induce modifications in cMCP-1 in this control male population, we constructed a multivariate model including age, BMI, WHR, hemoglobin

A_{1c}, and S_i as independent variables. Only WHR was found to be associated with cMCP-1 ($B = 1.42, p = 0.007$).

Adipose Tissue Gene Expression Study

The characteristics of the non-diabetic and type 2 diabetic populations are shown in Table 2. Age and sex were included as confounding variables in all of the analyses performed.

Non-diabetic Subjects. MCP-1 mRNA levels were found to be higher in the non-severely and severely obese compared with lean subjects ($p < 0.05$; Table 3). In fact, a positive correlation between BMI and MCP-1 mRNA expression was observed ($r = 0.41, p = 0.003$; Figure 1A). MCP-1 mRNA significantly correlated with TNF- α mRNA and TNFR2 mRNA as inflammatory markers (Figure 1B and C; Pearson bivariate correlation: $r = 0.42, p = 0.002$ and $r = 0.41, p = 0.003$, respectively). In a multiple

Table 3. Relative mRNA levels in subcutaneous adipose tissue

	Non-diabetic	Type 2 diabetes
MCP-1		
Lean	7.64 ± 0.35	8.04 ± 0.45*
Non-severely obese	8.05 ± 0.45	8.38 ± 0.58*
Severely obese	8.35 ± 0.63†	8.40 ± 0.48
TNF-α		
Lean	5.47 ± 0.40	5.62 ± 0.22
Non-severely obese	5.61 ± 0.38	5.61 ± 0.38
Severely obese	6.04 ± 0.57‡	5.98 ± 0.47§

MCP-1, monocyte chemoattractant protein-1; TNF, tumor necrosis factor. Non-diabetic: *n* = 14, *n* = 26, and *n* = 11 for lean, non-severely obese, and severely obese groups, respectively.

Type 2 diabetes: *n* = 15, *n* = 26, and *n* = 15 for lean, non-severely obese, and severely obese groups, respectively.

* *p* < 0.05, type 2 diabetes vs. control.

† *p* < 0.05 compared with lean and non-severely obese.

‡ *p* < 0.02 compared with lean and non-severely obese.

§ *p* < 0.025 compared with lean.

regression model, after controlling for age, sex, BMI, and cMCP-1, we found that only TNF-α mRNA remained an independent determinant of MCP-1 mRNA levels (*B* = 0.43, *p* = 0.037).

TNF-α mRNA was found to be significantly up-regulated in severely obese compared with non-severely obese and lean subjects (*p* < 0.02; Table 3).

Regarding CD68 mRNA expression in subcutaneous adipose tissue, no significant correlation with obesity was observed (data not shown). However, a positive correlation was observed between CD68 mRNA levels and circulating soluble TNFR2, mainly in women (*r* = 0.31, *p* = 0.04).

Circulating MCP-1 levels were found to be associated with WHR (*r* = 0.322, *p* = 0.04); this association remained after controlling for age, sex, and BMI (*B* = 1.55, *p* = 0.014).

Type 2 Diabetic Patients. A positive significant correlation was observed between MCP-1 mRNA expression and TNF-α mRNA (*r* = 0.52, *p* < 0.0001; Figure 2A). No differences were found in MCP-1 mRNA expression between obesity groups in patients with type 2 diabetes (Table 3). However, MCP-1 mRNA was significantly higher in type 2 diabetic lean and non-severely obese subjects compared with their non-diabetic counterparts (*p* = 0.05; Table 3).

In a regression model, with MCP-1 mRNA as the dependent variable, we found that MCP-1 mRNA remained strongly associated with TNF-α (*B* = 0.62, *p* = 0.001).

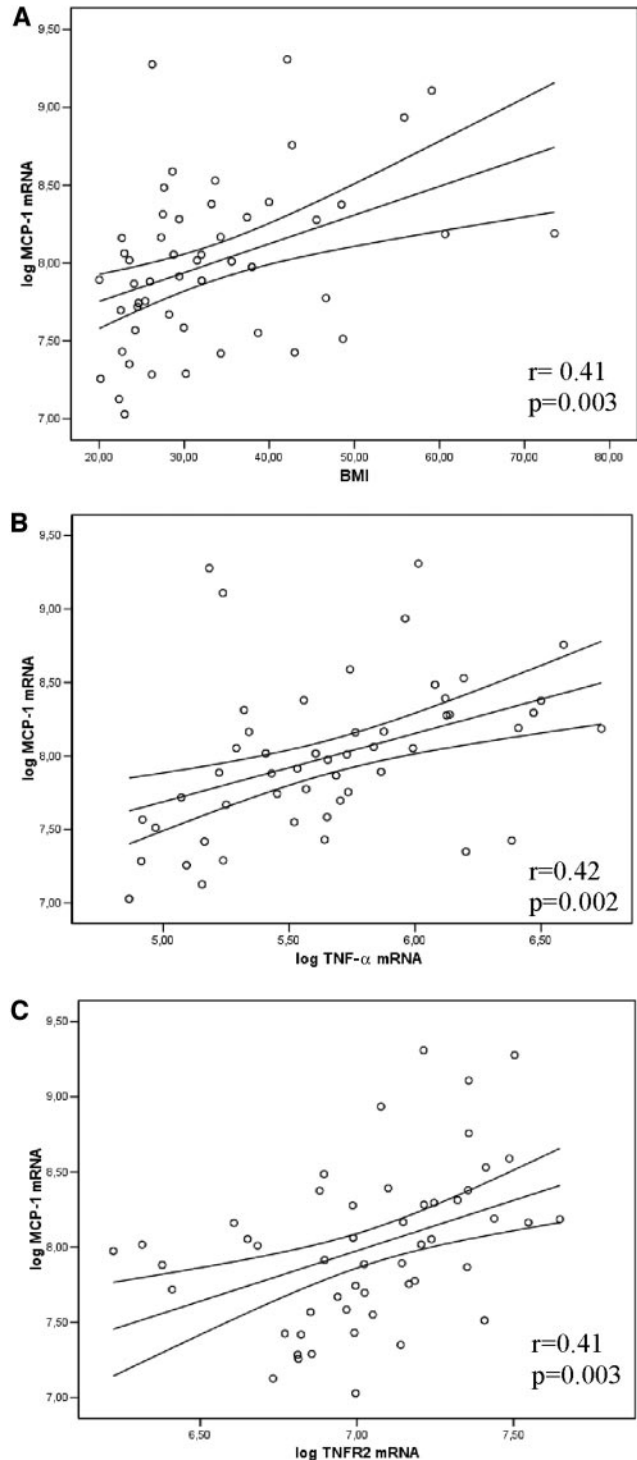


Figure 1: Correlations of MCP-1 mRNA in non-diabetic subjects.

A significant positive association between CD68 expression and BMI was observed in type 2 diabetic patients (*r* = 0.44, *p* = 0.001; Figure 2B) and remained as the only independent determinant of CD68 expression (*B* = 0.026, *p* = 0.004) after adjusting for age and sex.

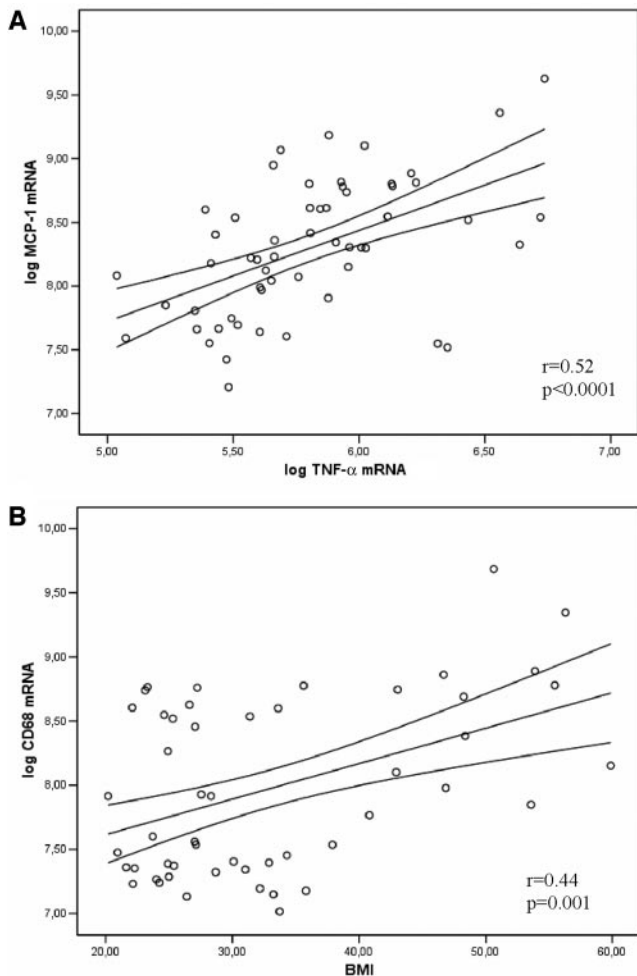


Figure 2: Correlations of MCP-1 mRNA in type 2 diabetic patients.

TNFR2 expression levels did not change between obesity groups or with the presence of type 2 diabetes (data not shown).

In a multiple regression model, after controlling for age, sex, and BMI, cMCP-1 was weakly associated with glucose ($B = 0.024$, $p = 0.046$).

Discussion

This study does not support a major role of cMCP1 on insulin sensitivity in men. The results of mRNA adipose tissue expression confirms a greater MCP-1 mRNA expression in obesity and in patients with type 2 diabetes.

The question about the role of MCP-1 in the pathogenesis of insulin resistance has not been answered yet. The inhibition of insulin-stimulated glucose uptake observed in the adipocytic cell line 3T3-L1 after chronic incubation with MCP-1, is the unique evidence of a direct participation of MCP-1 interfering with glucose metabolism (9). This find-

ing would be in agreement with the weak but positive correlation between hemoglobin A_{1c} and cMCP-1 observed in our control male cohort or with the association found between cMCP-1 and WHR (a well-known marker of increased cardiovascular risk) in non-diabetic subjects observed in the adipose tissue expression study. However, these are only weak indirect evidence in favor of a possible relation of this cytokine in glucose metabolism. In contrast, in the insulin sensitivity study performed here in a large number of control men, we observed an absence of association between cMCP-1 and S_i. This result is in accordance with a previous report in which insulin sensitivity was measured with a less robust method. In this report, in which MCP-1 circulating levels were analyzed and insulin resistance determined by homeostasis model assessment of insulin resistance in a large white cohort, many cardiovascular risk factors were found associated with higher levels of MCP-1 in univariate analysis. However, only age and high-density lipoprotein-cholesterol remained associated after controlling for several confounding factors (15). Similarly, in a large cohort of patients with type 2 diabetes, no association between serum MCP-1 levels and circulating insulin was observed (16). All these observations are in line with a recent study in which it was reported that there was no association between cMCP-1 levels and impaired glucose tolerance or type 2 diabetes (17).

It is difficult to extrapolate the in vivo observations about cMCP-1, its relationship with pro-inflammatory and insulin resistance markers, and its local regulation in adipose tissue in obesity and type 2 diabetes. Published evidence supported a role of MCP-1 in the atherosclerotic process (18,19). The circulating levels and its adipose expression may be modulated by several insulin-sensitizing mechanisms, such as treatment with rosiglitazone (20–23), exercise, and weight loss (24), but a direct participation in the insulin resistance process in humans remains to be shown.

Obesity is characterized by a broad inflammatory response, and inflammatory and stress-response genes are among the most abundantly regulated gene sets in adipose tissue (5). Adipose tissue in obese people is characterized by macrophage infiltration, and increased levels of MCP-1 in response to TNF- α would perpetuate this inflammatory response (5,25). MCP-1 expression is increased in adipose tissue of obese mice models (9,26,27) and, as we have shown here, in human subjects. The design of our study does not allow us to find the role of MCP-1 in the pathogenesis of obesity, and these findings may be only a consequence of a greater inflammatory infiltrate observed in obese people. A recent study showed that short-term treatment with a pharmacologic antagonist of chemokine receptor 2 (a high-affinity receptor for MCP-1) lowered macrophage content of adipose tissue and improved insulin sensitivity without significantly altering body mass in mouse models of obesity (28). This observation sets out a

new perspective in the mechanisms that involves MCP-1 in the process of obesity-related insulin resistance.

The results of our study showed a positive correlation between MCP-1 expression and BMI in non-diabetic subjects. No correlation was observed between MCP-1 mRNA expression and CD68 mRNA levels in subcutaneous adipose tissue. This absence of relationship can be interpreted in the sense that MCP-1 is coming from both macrophages and adipocytes, whereas CD68 is mainly produced by macrophages. These findings are in agreement and reinforce previous published data obtained from smaller cohorts of obese subjects (11–13). In type 2 diabetic patients, MCP-1 mRNA expression did not change with adiposity, but we found significantly higher expression in lean and non-severely obese patients compared with their non-diabetic counterparts. These results suggest that type 2 diabetes may have some additional influence in adipose tissue expression of MCP-1 with independence of the individual BMI.

The role of this adipose tissue overexpression remains unclear. Some authors have found an association between MCP-1 adipose expression and circulating levels of MCP-1 (12), suggesting that adipose tissue may be an important contributor to plasmatic MCP-1 levels. Our data do not confirm this observation; cMCP-1 plasma levels showed no direct correlation with adiposity in agreement with a very recent report in which the export of MCP-1 from adipose tissue to peripheral circulation was similar in obese and in lean subjects and was not affected by sex, age, and homeostasis model assessment of insulin resistance index (13). However, we cannot discard a relationship between visceral adipose tissue content and circulating MCP-1 levels. In fact, a positive relationship between WHR and cMCP-1 was observed in non-diabetic subjects of both studied cohorts.

A paracrine role of MCP-1 in adipose tissue has also been proposed, increasing the recruitment capacity of monocytes, hereby contributing to the observed macrophage infiltration of adipose tissue in obesity (25). In this sense, the close relationship observed in our work, between the expression of the pro-inflammatory cytokine TNF- α and its receptor TNFR2 with MCP-1 mRNA expression in the non-diabetic population, argues in favor of this theory.

In conclusion, our in vivo study argues against a direct relationship between cMCP-1 levels and insulin sensitivity, at least in control male subjects. Increased BMI is a determinant of a higher mRNA MCP-1 adipose tissue expression in non-diabetic subjects with a very close relationship with subcutaneous TNF- α gene expression. Considering a similar obesity degree, type 2 diabetic patients have proportionally higher amounts of MCP-1 subcutaneous adipose tissue expression. We need to stress the fact that MCP-1 has been analyzed only at gene expression level in subcutaneous adipose tissue samples and that protein levels need further analysis.

Finally, no association between subcutaneous adipose tissue *MCP-1* gene expression and plasma circulating MCP-1 levels was found.

We hypothesize that MCP-1 gene expression in adipose tissue may be regulated by paracrine–intracrine mechanisms and that sources other than adipose tissue may be contributing to cMCP-1 levels.

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