Inflammation may modulate IL-6 and C-reactive protein gene expression in the adipose tissue: the role of IL-6 cell membrane receptor

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Memoli B, Procino A, Calabrò P, Esposito P, Grandaliano G, Pertosa G, Del Prete M, Andreucci M, Di Lillo S, Ferulano G, Cillo C, Savastano S, Colao A, Guida B. Inflammation may modulate IL-6 and C-reactive protein gene expression in the adipose tissue: the role of IL-6 cell membrane receptor. *Am J Physiol Endocrinol Metab* 293: E1030–E1035, 2007. First published July 24, 2007; doi:10.1152/ajpendo.00697.2006.—Only few studies have been addressed to the presence and regulation of C-reactive protein (CRP) gene expression in different districts of adipose tissue, and no study has investigated the role of adipose tissue in presence of inflammation. Therefore, the aim of this study was to investigate the inflammatory involvement of either adipose tissue or adipose cells (adipocytes and stromal cells, respectively) in patients with chronic inflammatory disease, focusing on regional adipose tissue CRP gene expression. Eighteen patients with inflammatory disease and 14 healthy controls were enrolled. All subjects underwent specific surgical procedures. Inflamed and noninflamed patients provided samples of subcutaneous and/or omental adipose tissue. All samples were analyzed by RT-PCR and real-time PCR for specific gene expression. In addition, both adipocytes and stromal cells were studied by real-time PCR and immunoprecipitation to evaluate either gene or protein expression of CRP. Our results (real-time PCR) demonstrated a higher gene expression of CRP, IL-6, and both IL-6 membrane receptors in subcutaneous samples of inflamed patients than in healthy controls. Furthermore, in omental fragments of inflamed patients, an enhanced mRNA abundance of the same genes, compared with subcutaneous, was observed. The results obtained at cellular level did not provide evidence of any difference between adipocytes and stromal cell CRP gene expression, whereas immunoprecipitation demonstrated the presence of CRP in inflamed subjects. These results provide first-time evidence of the involvement of adipose tissue in the course of chronic inflammatory diseases, with a different degree of participation of the different adipose tissue districts.

interleukin-6; interleukin-6 receptors

CENTRAL ADIPOSE TISSUE is currently recognized (5, 20) as a rich milieu and source of interleukin-6 (IL-6). Circulating levels of IL-6 may be increased in obese individuals and linked with coronary artery disease (15, 16, 27). Studies measuring arterovenous increase of serum IL-6 (11) have clearly shown a net secretion of IL-6 by adipose tissue depots, suggesting that fat accounts for roughly 30% of circulating IL-6 in humans. In analogy with leptin secretion, IL-6 production by adipose tissue increases with increasing adiposity, and circulating levels of IL-6 are positively correlated with percent body fat (7) and insulin resistance (2).

IL-6 is the major regulator of the hepatic acute-phase protein response during inflammation. In humans, IL-6 causes a dramatic increase in synthesis (1,000-fold or more) of the two major acute-phase proteins, C-reactive protein (CRP) and serum amyloid A (23). Although the liver has been assumed as the only source of CRP synthesis and release, this protein has been demonstrated (3) to be produced also in other districts, such as the vascular walls. Data of the third National Health and Nutrition Examination Survey have shown (6, 10, 17, 18, 24) that CRP levels and body mass index (BMI) are strongly and positively associated each with other and with cardiovascular diseases. These findings may be relevant to explain the cardiovascular disease risk in obese subjects.

Ouchi et al. (13) first demonstrated a CRP gene expression in human subcutaneous adipose tissue; these results have been confirmed by Anty et al. (1), who have also demonstrated a CRP gene expression in omental fragments of adipose tissue drawn from obese subjects. Anty et al. did not find a statistical difference in CRP gene expression, when evaluating by real-time-PCR, between subcutaneous and omental adipose tissue biopsies. More importantly, they demonstrated that IL-6 may play an important role in the regulation of CRP gene expression in adipose tissue.

A positive effect of human recombinant IL-6 has recently been demonstrated by Calabrò et al. (4) on CRP synthesis and release by human adipocytes in culture. Taken together, these findings seem to support the idea that adipose tissue directly increases circulating levels of positive acute-phase proteins, such as CRP, under the stimulation of IL-6.

The effects of IL-6 on target cells occur via a complex receptor system composed of a ligand-binding subunit (IL-6R or gp80) and a signal-transducing glycoprotein (gp130), both expressed on cell surface (22). After IL-6 binding to IL-6R, the IL-6/IL-6R complex triggers the dimerization of the signal-transducing receptor component gp130. This receptor-ligand interaction activates Janus-activated kinases that phosphorylate tyrosine residues of the cytoplasmic portion of gp130, thereby activating a variety of members of signal transducers and activators of the transcription family (8, 21).

We (9) have recently demonstrated a positive role of inflammation in inducing a greater gene expression of IL-6, gp80, and gp130 in peripheral blood mononuclear cells harvested from uremic patients undergoing dialysis treatment. We have also demonstrated, by confocal microscopy, that the enhanced gp130 gene expression was positively correlated with an increased gp130 receptor expression at cell membrane level.

Although a specific association between the obesity and a low-grade chronic inflammation has been identified (28), the responses of adipose tissue to chronic inflammatory diseases and the role of adipose tissue-derived inflammatory factors in the course of a systemic inflammation still remain unknown.

The finding that circulating IL-6 may increase CRP synthesis and release by adipocytes (4) suggests a role of systemic inflammation in "activating" this tissue. Thus, the adipose tissue might be not only responsible for generating an inflammatory state per se but also by itself a target of a systemic inflammation by increasing the production of CRP. These considerations may have particular relevance in obese patients affected by chronic and/or degenerative diseases.

Therefore, this study has been addressed to evaluate the involvement of adipose tissue in patients with systemic chronic inflammatory diseases, focusing on the difference in regional adipose tissue CRP gene expression. The second aim was to identify the cell types involved in gene expression of CRP in the two different types of adipose tissue, the respective abundance of CRP mRNA and the presence of the CRP in these different cells.

METHODS

Patient and healthy control selection and adipose tissue samples. Thirty-two subjects were enrolled in this study. A first group of 20 subjects was divided in two subgroups on the basis of a preliminary evaluation of CRP circulating levels (assuming a cutoff level of 3 mg/l): *1*) eight normal healthy subjects, without any clinical symptoms or sign of inflammation, who underwent plastic surgery and *2*) 12 patients with chronic inflammatory disease who underwent elective surgical procedures: *a*) six for arthroprothesis, as treatment of coxarthrosis, and *b*) six patients operated for colon-rectal cancer.

To evaluate which cell type (adipocyte and/or stromal cells) is involved in inflammatory response, we successively enrolled, on the same basis of CRP concentration, a second distinct group of 12 subjects who were in turn divided into two subgroups: *1*) six healthy, noninflamed subjects (4 operated by laparoscopy of cholecystectomy and 2 of inguinal hernia, in absence of any symptom of acute or chronic illness) and *2*) six patients with colon cancer.

To avoid a possible confounding inflammatory effect exerted by obesity or overweight status, inflamed patients and healthy controls were BMI matched in both groups of subjects (Table 1). The presence of other systemic diseases (vasculitis, rheumatoid arthritis, osteoarthritis, and bowel or lung inflammatory disease) was excluded in all subjects; in particular, before the definitive inclusion, the possible existence of any immunological disease, malignancy (in the healthy group), and infectious disease was carefully investigated and excluded. No patient was diabetic and none assumed steroid or immunosuppressive therapy. All enrolled subjects provided written, informed consent, and the ethics committee of our hospital approved the study.

Blood samples were collected, before surgical procedures, from all studied subjects to obtain serum aliquots. Sera were stored at $-80°C$ until CRP and IL-6 assays were performed. Adipose tissue biopsies were obtained during the surgical procedures in the first 20 patients: 14 samples of subcutaneous total white adipose tissue (8 samples from *group 1* and 6 samples from *group 2A*) and six samples of omental adipose tissue from *group 2B*. In each one of the 12 additional subjects, either subcutaneous or omental adipose tissue fragments were obtained during surgical procedures.

After removal, to get rid of tissue debris the bioptic material was washed twice with warm sterile 0.9% NaCl solution; then the sample was immediately frozen in liquid nitrogen and stored at $-80°C$ until RNA extraction.

CRP and IL-6 assays. CRP circulating levels were determined by a high-sensitivity ELISA assay (Bender MedSystems, Vienna, Austria) on serum aliquots of all subjects included in the study. The lower detection limit was 3 pg/ml, and the overall intra-assay coefficient of variation has been calculated to be 6.9%. The concentrations of IL-6 in plasma samples were evaluated by ELISA using a commercially available kit (Quantikine; R&D Systems, Minneapolis, MN) as described elsewhere (9). The lower detection limit of IL-6 assay was <0.70 pg/ml, and the coefficient of variation of both inter- and intra-assay was <5%. All samples were analyzed in duplicate.

Identification of primers for IL-6, CRP, gp80, gp130. The genome sequences corresponding to the IL-6, CRP, gp80, and gp130 were obtained from GeneBank (www.ncbi.nlm.nih.gov) to identify specific primers. We analyzed the exon sequences to establish, for each gene, a pair of primers (sense and antisense) able to generate amplified fragments measuring between 150 and 700 bp in length, with annealing temperature between 55 and 62°C. The sense and antisense primers were selected to include at least one intron to prevent genomic DNA contamination during amplification. The selected primers are shown in Table 2.

RNA extraction from adipose tissue and expression analysis by means of RT-PCR. Frozen tissues were pulverized with a blender and lysed in guanidinium isothiocyanate. Total RNA was extracted by the single-step method, using phenol and chloroform/isoamylalcohol. Four micrograms of total RNA was subjected to cDNA synthesis for 1 h at 37°C using the "Ready to Go You-Primer First-Stand Beads" kit (code. no. 27-9264-01; Amersham Pharmacia Biotech, Little Chalfont, UK) in a reaction mixture containing 0.5 µg oligo(dT) (code. no. 27-7610-01; Amersham Pharmacia Biotech).

Table 1. *Demographic and anthropometric characteristics of all subjects studied*

	Healthy Subjects	Chronic Inflamed Patients	P Value
n	14	18	
Sex (M/F)	7/7	10/8	
Age, yr (range)	43.7±4.0 (36–50)	41.9±3.9 (33–48)	NS
Body weight, kg (range)	73.9±11.0 (61.0–88.0)	79.5±11.3 (62.0–91.0)	NS
Body mass index, kg/m² (range)	25.9±1.8 (24.0–28.2)	26.1±1.3 (23.8–29.0)	NS
Waist circumference, cm (range)	91.8±4.9 (83–102)	91.3±5.9 (78–103)	NS
Waist-to-hip ratio (range)	0.79±0.1 (0.54–1.0)	0.84±0.1 (0.56–1.1)	NS
CRP, mg/l (range)	2.180±0.540 (1.6–3.0)	7.260±3.267 (4.1–14.6)	P < 0.01
IL-6, pg/ml (range)	2.85±1.35 (1.6–5.6)	37.06±10.82 (18.8–54.6)	P < 0.01

Values are means ± SD; n = 32 subjects. M, male; F, female; NS, not significant; CRP, C-reactive protein.

Table 2. *Oligonucleotide sequences designated for this study*

Primers	Sense	Antisense
CRP	TTTCTTCGTCTTGACCAGCC	TTCTTCAGACTCTTCCTCACCC
gp80	CATTGCCATTGTTCTGAGGTT	AGTAGTCTGTATTGCTGATGT
gp130	CATGCTTTGGGTGGAATGGAC	CATCAACAGGAAGTTGGTCCC
IL-6	ATGTAGCCGCCCCACACAGA	GCATCCATCTTTTTCAGCCATC
β-Actin	CACCATGGATGATGATATCG	TGGATAGCAACGTACATGG

PCR amplification of cDNA was performed in a reaction mixture containing 5 µl of cDNA sample and different primer sets (20 pmol each). The coamplification of each gene and human β-actin gene, as an internal control, was achieved using two primer sets in a single reaction mixture. We selected a pair of β-actin primers to obtain amplified fragments measuring 450 bp (see Table 1). PCR products were separated by ethidium 1.2% agarose gel electrophoresis.

Isolation of adipocytes and stromal cells from adipose tissue fragments. Adipose tissue fragments obtained from the 12 additional subjects were placed in 37°C, sterile 0.9% NaCl, 5.6 mM glucose, and 25 mM HEPES buffer with pH adjusted to 7.4 and containing 50 U penicillin/ml plus 50 mg streptomycin/ml. The adipose tissue fragments were minced under sterile conditions and digested in Krebs-Ringer bicarbonate buffer supplemented with 5.6 mM glucose, 50 U penicillin/ml, 50 mg streptomycin/ml, and 17 mg type I collagenase/10 g adipose tissue (Worthington Biochemical, Lakewood, NJ). Digestion was for 75 min at 37°C, with rotary agitation at 40 rpm. The isolated cells were filtered through a single layer of chiffon, and the isolated adipocytes were allowed to float for 5 min at 37°C. The fluid under the floating adipocytes (containing stromal fraction) was transferred to a conical polypropylene 50-ml centrifuge tube. For the adipocytes, 1 ml of cell suspension of adipose cells was suspended in each tube containing specific adipocyte medium (Zen-Bio, Research Triangle Park, NC) and incubated in the specific conditions until the lysis process. The stromal fraction was centrifuged at 800 g for 10 min, and the cell pellet was immediately lysated as described below.

Cell lysate preparation for real-time-PCR. RNA extraction with Trizol reagent was performed according to the manufacturer's instructions. Both adipocytes and stromal fraction were separately minced and homogenized after adding 0.5 ml of Trizol reagent (GIBCO-BRL) at 4°C. Each homogenate was moved to another tube before 0.1 ml of chloroform was added. After that, the tube was maintained for 3 min at 0°C. This was followed by a 15-min centrifugation at 12,000 g at 4°C. The upper aqueous phase was removed and transferred to a fresh tube for RNA preparation; subsequently, 0.25 ml of isoamyl alcohol was added to aqueous phase. The mixture was placed for 5 min at room temperature and then centrifuged at 12.000 g for 10 min at 4°C. The RNA sample was then precipitated with 0.5 ml of 75% ethanol and collected by centrifugation at 7.500 g for 5 min. The RNA was dissolved in 30 µl of DEPC-H$_2$O and immediately stored at −70°C. The concentration and the purity of RNA were evaluated by measuring the absorbance at 260 nm and calculating the ratio of absorbance at 260–280 nm using a UV spectrophotometer (DU-800 spectrophotometer; Beckman Instruments).

Real-time PCR. Real-time quantitative PCR analysis for IL-6, CRP, gp80, and gp130 gene was performed on adipose tissue and cell lysate (both adipocytes and stromal fraction) using ABI prism 7500 (Applied Biosystems, Foster City, CA) and the 5′exonuclease assay (TaqMan technology). The cDNA, synthesized as described above, was used for real-time PCR performed in 96-well optical reaction plates with cDNA equivalent of 100 ng of RNA in a volume of 25-µl reaction containing 1× Taqman Universal Master Mix, optimized concentrations of FAM-labeled probe, and specific forward and reverse primer for IL-6, CRP, gp80, and gp130 selected from Assay on Demand (Applied Biosystems). Controls included RNA subjected to RT-PCR without reverse transcriptase and PCR with water replacing cDNA.

The results were analyzed using a comparative method, and the values were normalized to the β-actin expression and converted into fold change based on a doubling of PCR product in each PCR cycle according to the manufacturer's guidelines, as previously described (14).

Cell lysate preparation and immunoprecipitation. Isolated adipocytes and stromal cells obtained from both subcutaneous and omental adipose tissue fragments were washed twice with ice-cold PBS and lysed in situ in RIPA buffer (1 mM phenylmethylsulphonylfluoride, 5 mM EDTA, 1 mM sodium orthovanadate, 150 mM sodium chloride, 8 µg/ml leupeptin, 1.5% Nonidet P-40, 20 mM Tris·HCl, pH 7.4) for 30 min on ice. The cell lysate was centrifuged at 10,000 g for 5 min at 4°C. The supernatants were collected and stored at −80°C until they were used. Two hundred micrograms of proteins from the supernatant were immunoprecipitated using the protein G immunoprecipitation kit (Sigma, Milan, Italy). The proteins were first incubated with 2.5 µg of anti-CRP monoclonal antibody overnight on a rocking platform at 4°C and then with protein G-Sepharose for 3 h at 4°C. The immunoprecipitated proteins were eluted in sample buffer (2-β-mercaptoethanol, 10% SDS, 10% glycerol, 0.5 M Tris·HCl, pH 6.8, 0.05% blue-bromophenol), boiled, and separated by electrophoresis on a 10% polyacrylamide gel. The proteins in the gel were then electrotransferred onto nitrocellulose membrane (Hybond; Amersham). The membrane was blocked overnight at room temperature (rt) with 2% bovine serum albumin in PBS containing 0.1% Tween-20 (TBS) and incubated with anti-CRP monoclonal antibody (1:400; Santa Cruz Biotechnology, Santa Cruz, CA) for 2 h at rt, washed, and incubated with horseradish peroxidase-conjugated sheep anti-mouse IgG (1:1,500) for 1 h at rt. The enhanced chemiluminescence system was used for detection.

Statistical analysis. Statistical analysis was performed by using unpaired *t*-test and ANOVA (followed by Bonferroni post hoc test). Results are expressed as means ± SD; statistical significance was defined as $P < 0.05$.

RESULTS

Demographic, anthropometric, and biochemical baseline data of all enrolled subjects are reported in Table 1. No significant difference was observed in sex, age, body weight, BMI, waist circumference, and waist-to-hip ratio between the two different groups. On the contrary, both plasma CRP and IL-6 circulating levels were much higher in patients with chronic inflammatory diseases than in healthy subjects ($P < 0.01$; Table 1).

CRP, IL-6, gp80, and gp130 gene expression by RT-PCR in the adipose tissue. We first investigated, by RT-PCR, whether all districts of adipose tissue express the genes of CRP and all components of the IL-6 system (i.e., IL-6, gp80, and gp130).

Adipose tissue did express CRP (Fig. 1A); the mRNA for this marker was found in all fragments, i.e., in subcutaneous fragments of controls and in both subcutaneous and omental fragments of inflamed patients. Also, IL-6 mRNA was expressed in subcutaneous and omental fragments of adipose
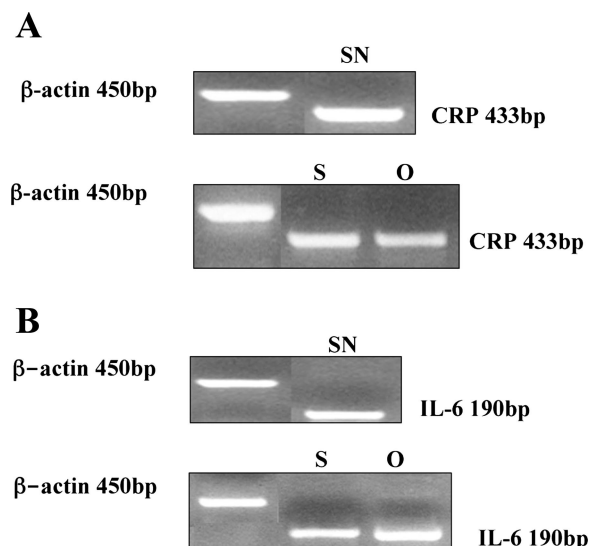
A

B

Fig. 1. C-reactive protein (CRP; *A*) and interleukin-6 (IL-6; *B*) gene expression obtained by RT-PCR in adipose tissue fragments from normal healthy subjects [subcutaneous normal (SN); *A* and *B*, *top*] and inflamed patients [subcutaneous (S) and omental (O); *A* and *B*, *bottom*]. β-Actin gene expression is also reported as housekeeping. Each image is representative of 5 experiments.

tissue from all subjects (Fig. 1*B*). Furthermore, a significant expression of both components of IL-6 receptor complex, gp80 and gp130 (Fig. 2, *A* and *B*, respectively), was demonstrated in all adipose tissue samples.

CRP, IL-6, gp80, and gp130 gene expression studied by real-time-PCR. In samples of subcutaneous adipose tissue obtained from inflamed patients, CRP gene expression was significantly increased (50-fold) compared with controls. CRP gene expression in omental samples of inflamed patients was also significantly higher than in subcutaneous samples of both controls (150-fold) and inflamed patients (100-fold; Fig. 3, open bars).
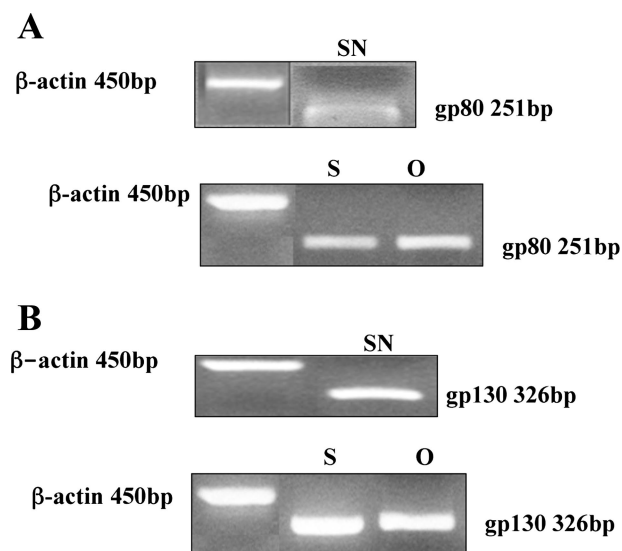
A

B

Fig. 2. gp80 (*A*) and gp130 (*B*) Gene expression obtained by RT-PCR in adipose tissue fragments from normal healthy subjects (SN; *A* and *B*, *top*) and inflamed patients (S and O; *A* and *B*). β-Actin gene expression is also reported as housekeeping. Each image is representative of 5 experiments.
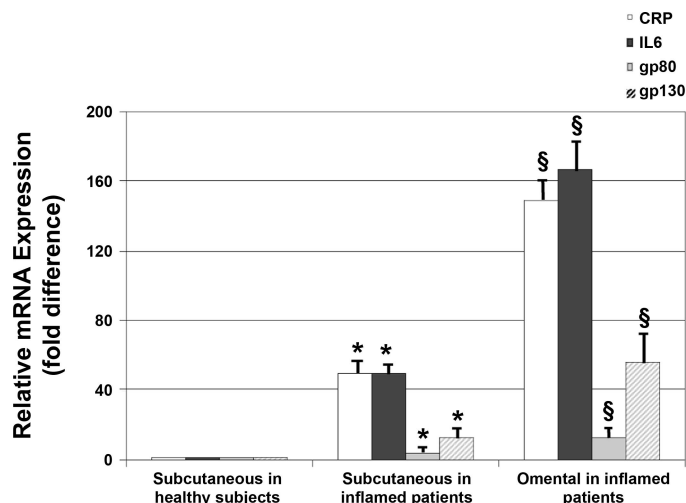

Fig. 3. Gene expressions of CRP, IL-6, gp80, and gp130 in different sites of adipose tissue, obtained by real-time PCR analysis. The columns show the relative expression of the mRNA abundance (of CRP, IL-6, gp80, and gp130, respectively) in subcutaneous adipose tissue fragments of healthy subjects compared with those obtained in both S and O adipose tissue of inflamed patients. The values on the *y*-axis represent arbitrary units derived from the mean expression value for each gene of inflamed patients (both S and O) compared with the values of the S adipose tissue of healthy noninflamed subjects set at 1. *$P < 0.01$ vs. healthy control; §$P < 0.01$ vs. S (both control subjects and inflamed patients).

The results concerning the IL-6 gene expression were similar; as shown in Fig. 3 (black bars), IL-6 gene expression was significantly higher (50-fold) in subcutaneous adipose tissue of inflamed patients than in healthy subjects. IL-6 gene expression in omental samples of adipose tissue of inflamed patients was much higher than in subcutaneous fragments of both healthy controls (167-fold) and inflamed patients (117-fold).

Figure 3 also shows the comparison of gp80 and gp130 gene expression in subcutaneous adipose tissue of both healthy subjects and inflamed patients and in omental adipose tissue of inflamed patients. Both gp80 and gp130 gene expressions were significantly higher (5-fold and 13-fold, respectively) in subcutaneous adipose tissue of inflamed patients than in healthy subjects; both gp80 and gp130 gene expressions in omental samples of adipose tissue of inflamed patients were higher than in subcutaneous fragments of both healthy controls (12-fold and 56-fold, respectively) and inflamed patients (8-fold and 44-fold, respectively) (Fig. 3).

CRP gene expression, studied by real-time-PCR, in different cell types extracted from specific sites of adipose tissue. Figure 4 shows the different gene expression of CRP, studied by real-time-PCR, in both adipocytes and stromal cells obtained from either subcutaneous or omental fragments of adipose tissue drawn from both noninflamed and inflamed subjects. A statistically significant difference was observed, both in inflamed and noninflamed subjects, between the values in adipose cells obtained from fragments of subcutaneous adipose tissue compared with those from omental fragments; the inflammation caused a further increase of CRP gene expression in both districts. No difference was evidenced between adipocytes and stromal cells.

CRP protein expression in the different cell types obtained by the different types of adipose tissue studied by immunoprecipitation. Finally, we investigated whether at the protein level both adipocytes and stromal cells were also able to express
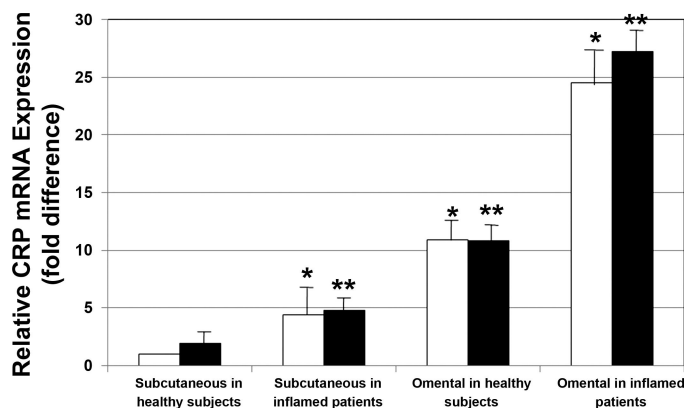
Fig. 4. Gene expression of CRP, studied by real-time PCR, in either adipocytes (open bars) or stromal cells (black bars) obtained in both noninflamed and inflamed subjects from either S or O fragments of adipose tissue. *$P <$ 0.01 vs. all adipocytes and stromal cells except vs. corresponding stromal cells; **$P <$ 0.01 vs. all adypocytes and stromal cells, except vs. corresponding adipocytes.

CRP in inflamed patients. As shown in Fig. 5, immunoprecipitation analysis demonstrated that adipocytes and stromal cells synthesize CRP at the same levels in subcutaneous as well as in visceral adipose tissue.

DISCUSSION

Our results clearly demonstrate that adipose tissue fragments, extracted from different districts (subcutaneous and omental) of noninflamed and inflamed patients, show a clear expression of both CRP and IL-6 mRNA (Fig. 1). When evaluated by real-time-PCR (Fig. 3), gene expression of both CRP and IL-6 was much higher in inflamed patients than in controls both in subcutaneous (50-fold for both) and in intra-abdominal (omental) adipose tissue (150-fold and 167-fold, respectively).

Several physiological and metabolic characteristics of adipose tissue are site specific. There is a large body of evidence that depot-specific differences also exist in the expression of genes coding important functional proteins in adipocytes (15, 16). Fried et al. (7) have shown that incubated fragments of omental adipose tissue release in the supernatant two to three times more IL-6 compared with fragments withdrawn from subcutaneous fat. These observations are in line with our results and suggest a different degree of activation of specific sites of adipose tissue (subcutaneous and intra-abdominal) during a systemic inflammation.

Our data confirm in part the results of Anty et al. (1), who have demonstrated a greater CRP gene expression in subcutaneous adipose tissue fragments of obese subjects (with higher circulating levels of CRP) compared with healthy control subjects; these authors, however, did not find a statistically significant difference in CRP gene expression between visceral and subcutaneous adipose tissue (visceral CRP gene expression was, however, 4 times greater than the subcutaneous one).

Which type of cell of the adipose tissue is devoted to IL-6 and CRP production is still a matter of discussion. It has been hypothesized that macrophages infiltrating fat tissue represent the principal site of obesity-related cytokine synthesis (25). Conversely, according to Fried et al. (7) and Wisse (26), the same amount of IL-6 is expressed by adipocytes, macrophages, and stromal vascular cells.

Our results, focused on CRP gene expression, have clearly demonstrated a greater abundance of CRP mRNA in the cells obtained from omental than from subcutaneous fragments and values higher in inflamed patients than in noninflamed. On the contrary, no difference was evidenced between the results obtained in adipocytes compared with the results obtained in stromal cells (Fig. 4). Anty et al. (1), who have evaluated the CRP gene expression only in cells obtained from subcutaneous adipose tissue, have observed a higher gene expression in stromal cells compared with adipocytes. The discrepancy between our results and those of Anty et al. could be explained by the different type of recruited subjects; the subjects enrolled by Anty et al., in fact, are severely obese (BMI range: 42–47), and it is well known that in obesity a greater infiltration of macrophages in the adipose tissue does occur with a major inflammatory condition within the stromal fraction. Concerning this important issue, recent studies have also demonstrated the importance of CC motif chemokine receptor-2 in macrophage recruitment in adipose tissue of obese patients (12). Our results are confirmed by the data of CRP protein expression; the immunoprecipitation analysis, in fact, demonstrated the presence of this protein in both adipocytes and stromal cells obtained from both subcutaneous and omental adipose tissue fragments in inflamed subjects, without an appreciable difference between these two cell types (Fig. 5).

A further, important result of this study is the demonstration that adipose tissue fragments from distinct sources (subcutaneous and omental) express mRNA of both components of the IL-6 cell membrane receptor system, gp80 (or IL-6R) and gp130 (Fig. 2), and that this gene expression abundance is enhanced by the presence of a systemic inflammatory state (Fig. 3). In other words, gene expression of both gp80 and gp130 receptors is increased (over 5- and 13-fold, respectively) in fragments of subcutaneous adipose tissue obtained from inflamed patients compared with healthy control subjects. In addition, in inflamed patients, the omental expression is even greater than the subcutaneous both in healthy ($>$12- and 56-fold, respectively) and in inflamed patients ($>$8-fold and 44-fold, respectively). These results are in agreement with our previous results (9) obtained in peripheral blood mononuclear cells; in these cells, in fact, the inflammatory state induced a greater gene expression of both IL-6 receptors followed by their greater synthesis and expression on the cell surface, as demonstrated by confocal microscopy.
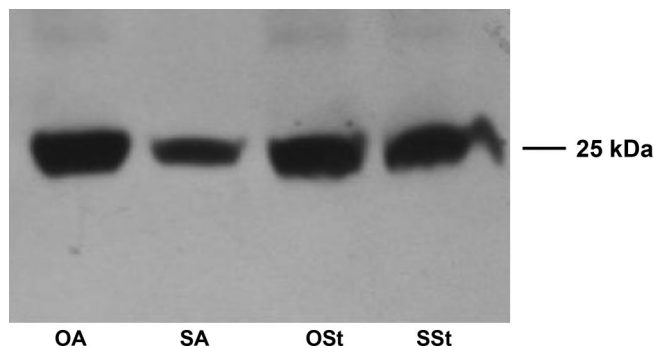

Fig. 5. CRP expression in O and S adipocytes (A) and stromal cells (St) in a representative inflamed patient. Immunoprecipitation of CRP was performed as described in METHODS. CRP expression was detectable at the same levels in both A and St. The image is representative of 5 experiments.

The results of the present study, therefore, suggest that in the adipose tissue, like in the liver, the synthesis of CRP is modulated by IL-6, following the binding of this ligand with its specific cell membrane receptor system composed of one chain of gp80 (or IL-6R) and two chains of gp130. These findings agree with those of Anty et al. (1), who have demonstrated that IL-6 may upregulate CRP gene expression in human adipose tissue.

Taken together, our results demonstrate that inflamed patients exhibit an increasing expression of CRP, IL-6, gp80, and gp130 in subcutaneous and, particularly, in omental adipose tissue compared with noninflamed healthy subjects. The higher circulating levels of IL-6 and other proinflammatory cytokines may induce, in these patients, a greater CRP and IL-6 gene expression and, presumably, a greater CRP and IL-6 synthesis and release by both cell components (adipocytes and stromal cells, respectively) of adipose tissue. This hypothesis is strongly supported by the results recently obtained by Calabrò et al. (4), who have demonstrated an IL-6-induced positive modulation of CRP release by cultured adipocytes. Interestingly, the authors also demonstrated that treatment with several anti-inflammatory drugs, such as aspirin, troglitazone, and fluvastatin, induced a reduction of CRP release from adipocytes. These data could in part explain the beneficial effects of selected drugs (particularly statins) in reducing CRP circulating levels (19). Therefore, our findings, which are focused on the same issue, may suggest new perspectives to reduce global cardiovascular risk, particularly in obese and inflamed subjects.

In conclusion, adipose tissue and its cells (both adipocytes and stromal) seem deeply involved in systemic inflammation since they may upregulate the mRNA of CRP, IL-6, and IL-6 receptors, thereby amplifying inflammation. Thus our results suggest a potential new action of the adipose tissue as inflammatory organ and strengthen its role in the inflammatory response induced by chronic systemic diseases.

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