"In vitro hyper-responsiveness to TNF-alpha contributes to adipokine dysregulation in omental adipocytes of obese subjects."

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ABSTRACT

Context: In obesity, adipocyte hypertrophy and macrophage infiltration lead to overproduction of proinflammatory adipokines, which play a crucial role in the metabolic syndrome. The molecular mechanisms underlying this overproduction are still unsettled. The role of TNF-alpha also remains controversial in human obesity. Objective: We revisited the contribution of TNF-alpha to adipokine dysregulation in central obesity. We more particularly assessed the involvement of TNF-alpha vs. other stromal-vascular cell (SVC)-secreted factors and searched for potential differential responses to TNF-alpha between adipocytes of lean and obese individuals. Design and participants: Primary cultures of omental adipocytes from obese and non-obese age- and sex-matched subjects were used. For some experiments, we generated media previously conditioned by SVC, which mimic adipocyte microenvironment. Results: Adipocytes of obese subjects mainly overexpressed adipokines, in comparison to those of lean ones...

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In Vitro Hyperresponsiveness to Tumor Necrosis Factor-α Contributes to Adipokine Dysregulation in Omental Adipocytes of Obese Subjects

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Context: In obesity, adipocyte hypertrophy and macrophage infiltration lead to overproduction of proinflammatory adipokines, which play a crucial role in the metabolic syndrome. The molecular mechanisms underlying this overproduction are still unsettled. The role of TNF-α also remains controversial in human obesity.

Objective: We revisited the contribution of TNF-α to adipokine dysregulation in central obesity. We more particularly assessed the involvement of TNF-α vs. other stromal-vascular cell (SVC)-secreted factors and searched for potential differential responses to TNF-α between adipocytes of lean and obese individuals.

Design and Participants: Primary cultures of omental adipocytes from obese and nonobese age- and sex-matched subjects were used. For some experiments, we generated media previously conditioned by SVCs, which mimic adipocyte microenvironment.

Results: Adipocytes of obese subjects mainly overexpressed adipokines, in comparison with those of lean ones, when cultured in SVC-conditioned media. This was abrogated by immunoneutralization of TNF-α, indicating that among the numerous factors secreted by SVCs, TNF-α is a crucial contributor to adipokine dysregulation. Accordingly, adipocytes of obese subjects overproduced adipokines in response to direct exposure of TNF-α. This hyperresponsiveness was mediated by TNF-α receptor 1 and hyperactivation of the nuclear factor-κB (NF-κB) pathway. Correspondingly, NF-κB activity was increased in adipocytes of obese subjects and correlated with adipocyte size, adipokine expression, and in vivo insulin resistance. Eventually adipokine overexpression in adipocytes of obese subjects was prevented by NF-κB inhibitors.

Conclusions: In obesity, TNF-α, i.e. over other SVC-secreted factors, a crucial determinant of adipokine dysregulation acts on enlarged adipocytes, which are hyperresponsive to this triggering signal. This ultimately exacerbates adipokine production, inflammation, and the metabolic syndrome. (J Clin Endocrinol Metab 94: 1393–1400, 2009)

In increased adiposity leads to local inflammation as a consequence of adipocyte hypertrophy and associated infiltration of macrophages. Both events cause dysregulation of adipokine production with oversecretion of deleterious adipokines and hyposecretion of defensive ones (1, 2). Such a dysregulation triggers the development of a low-grade proinflammatory state, which is considered to build the common soil for the development of obesity comorbidities and the metabolic syndrome (1, 3, 4). Preferential accumulation of omental rather than sc fat appears to be a stronger risk factor for this adverse health profile (1).

Previous work from our laboratory has identified six deleterious adipokines that are oversecreted by omental adipose tissue (OAT) in obesity: three chemokines [growth-related oncogen factor (GRO), macrophage inflammatory protein (MIP)-1β, regulated upon activation normal T cells expressed and secreted (RANTES)], three adipokines [homeostasis model assessment-estimated insulin resistance index (HOMA-IR), tissue inhibitor of metalloproteinase (TIMP), thrombopoietin (TPO)], and thrombopoietin.

Abbreviations: BMI, Body mass index; GRO, growth-related oncogen factor; HOMA-IR, homeostasis model assessment-estimated insulin resistance index; JNK, c-Jun NH2-terminal protein kinase; MIP, macrophage inflammatory protein; NF-κB, nuclear factor-κB; OAT, omental adipose tissue; RANTES, regulated upon activation normal T cells expressed and secreted; RTQ-PCR, real-time quantitative PCR; SVC, stromal-vascular cell; TIMP, tissue-inhibitor of metalloproteinase; TNFR, TNF-α receptor; TPO, thrombopoietin.
ulated upon activation normal T cells expressed and secreted (RANTES), one interleukin (IL-7), one tissue-inhibitor of metalloproteinases (TIMP)-1 and one megakaryocytic growth factor [thrombopoietins (TPO)] (5). Several pieces of evidence indicate that these adipokines may contribute to obesity-linked disorders. Thus, all of them may play a role in cardiovascular disease via different mechanisms, whereas some may be involved or associated with insulin resistance/type 2 diabetes (GRO, MIP-1 β, RANTES and TIMP-1) or worsen obesity itself (TIMP-1) (reviewed in Ref. 5). In our previous work, enhanced expression of these adipokines in adipocytes did correlate with several features of the metabolic syndrome (5), but the mechanisms underlying their overproduction in obesity are still unknown.

Obesity is associated with several endocrine abnormalities [in particular hyperinsulinemia, enhanced glucocorticoid turnover, and altered sympathetic tone (6, 7)]. The abnormal hormonal milieu may be involved in adipokine dysregulation (8–10), but the inflammatory state per se may also contribute (11). When we compared the relative contribution of hormones and TNF-α, we found that TNF-α was the most potent inducer of adipokine changes. Yet the role of TNF-α remains controversial in human obesity (12). The aim of the present work was therefore to revisit the contribution of TNF-α to adipokine dysregulation in human central obesity. We focused on the involvement of TNF-α vs. other stromal-vascular cell (SVC)-secreted factors and searched for potential differential responses to TNF-α between adipocytes of lean and obese individuals in vitro.

### Subjects and Methods

#### Subjects

OAT was obtained from obese and nonobese age- and sex-matched subjects undergoing abdominal surgery after an overnight fast (Table 1). Obesity was defined as a body mass index (BMI) of 30 kg/m² or greater. Subjects and Methods

<table>
<thead>
<tr>
<th>Nonobese</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>55.6 ± 2.3</td>
</tr>
<tr>
<td>Sex ratio (men/women)</td>
<td>17/11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.8 ± 0.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>121 ± 4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>Glucose homeostasis</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/liter)</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>Insulin (mU/liter)</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mmol/liter)</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Total cholesterol/HDL</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/liter)</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Adipokines and inflammation</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>10.3 ± 1.8</td>
</tr>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>10.0 ± 1.2</td>
</tr>
<tr>
<td>hsCRP (mg/liter)</td>
<td>7.0 ± 1.2</td>
</tr>
</tbody>
</table>

Clinical and laboratory parameters were obtained in the fasted state before surgery. Values are means ± SEM for 28 lean and 27 obese subjects. For cultures of adipocytes, due to the limited availability of OAT, not all data could be generated from all patients. However, when subgroups of obese and nonobese subjects were compared, each subgroup was always representative of the respective whole group (i.e. similar clinical/laboratory characteristics: BMI, age, sex ratio, insulin levels, etc.).

#### Culture of mature adipocytes freshly isolated from OAT

OAT biopsies were transported to the laboratory within 5–10 min after sampling (6). Samples were fractionated into adipocytes and SVC (that mainly contain macrophages, endothelial cells, and preadipocytes) by collagenase treatment, as reported (5, 6). This yields two high purity fractions (5). Adipocytes (1 ml packed cells) were cultured for up to 24 h, as described (5). Digestion and 24-h culture did not influence secretion and release of the studied adipokines (5).

For some experiments, SVC-conditioned media were generated. These media were collected after culturing SVC (500 μl per 3 g initial tissue) for 24 h, as described for adipocytes (5, 6). Aliquots of medium were stored at −20 °C and then mixed before use [2:1, (vol/vol)] with fresh medium to serve as conditioned medium, in which mature adipocytes were cultured.

In other experiments, human recombinant TNF-α (PreproTech, Rocky Hill, NJ), different antibodies directed against human TNF-α, human TNF-α receptor (TNFR) 1 or TNFR2 (R&D Systems, Minneapolis, MN), or protease inhibitors [nuclear factor-κB (NF-κB) inhibitor BAY11-7085, c-Jun NH2-terminal protein kinase (JNK) inhibitor SP600125, both from Calbiochem, La Jolla, CA] were added to the medium alone or in combination. The concentrations used were recommended by the manufacturer (antibodies) or similar to those reported by others (13, 14) and devoid of overt toxicity as assessed by measurement of low and unchanged release of lactate dehydrogenase in media and microscopical examination (6). At the end of the experiments, cells were frozen at −80 °C. Average adipocyte volume in a sample was calculated using lipid weight (Folch’s method), cell number [estimated by DNA
interleukin media were saved. Because similar results were obtained with differentiated adipocytes from either lean (n as relative expression compared with untreated cells. normalized to the levels of TATA box-binding protein (TBP; used as reporter gene) and presented picogram per microgram adipocyte DNA. Values are means quantification (8)] and the density of triolein (15). Mean adipocyte size (diameter) was derived from mean adipocyte volume assuming that cells are spheres.

**Culture of adipocytes differentiated in vitro from omental precursor stromal cells**

Omental preadipocytes were grown to confluence and then differentiated in vitro as described (Ref. 5, except that only 5 mM dexamethasone were used). A full stage of differentiation was achieved at d 12 (5), with was no morphological difference between adipocytes obtained from SVC of lean or obese subjects (not shown). Differentiated adipocytes were then cultured for 24 h without insulin and dexamethasone, which did not affect the expression of late adipocyte markers or the investigated adipokines, we focused thus, hormones caused only modest changes in the expression of adipokines by newly differentiated adipocytes.

**RNA extraction and real-time quantitative PCR (RTQ-PCR)**

Total RNA was extracted and reverse transcribed, and RTQ-PCR was performed with designed primers (supplemental Table 2), as described elsewhere (5). Relative changes in the expression level of one specific gene were presented as $2^{-\Delta\Delta Ct}$ (5).

**Quantification of adipokines in culture media**

Adipokines were quantified by specific ELISAs as described (5).

**NF-κB activity assay**

The TransAM NF-κB p65 transcription factor assay kit was used (Active Motif, Rixensart, Belgium). Two hundred microliters of adipocytes were thawed in the complete lysis buffer provided by the manufacturer (Active Motif, Rixensart, Belgium), and whole-cell extracts were prepared after removal of the lipid supernatant. Binding of p65 NF-κB transcription factors was detected by ELISA. Signal intensity of NF-κB activation was proportional to adipocyte proteins (5–30 μg); approximately 10–15 μg adipocyte proteins were used per well. Data were expressed as OD and normalized on a per cell basis (per microgram DNA).

**Results**

**Effects of hormones and TNF-α on adipokine production by newly differentiated adipocytes**

We first examined the hormonal regulation of the investigated adipokines in human omental adipocytes that were fully differentiated. Insulin, dexamethasone, or isoproterenol (a β-adrenergic agonist used as a surrogate of catecholamines) induced only moderate (less than 2-fold) increases in GRO and TPO mRNAs, whereas the glucocorticoid and the β-agonist also slightly decreased MIP-1β and/or IL-7 (supplemental Table 3). Thus, hormones caused only modest changes in the expression of some adipokines.

By contrast, TNF-α induced a strong rise in the expression of all the investigated adipokines (except for TPO) (Fig. 1). This rise occurred in a dose-dependent fashion and amounted to 600-fold of control values for some adipokines (i.e. RANTES). These changes in mRNA abundance were accompanied by parallel changes in secretion levels (Fig. 1, insets). Because TNF-α induced potent changes on the investigated adipokines, we focused on its effects.

**TNF-α production by OAT of lean and obese subjects**

We first verified that TNF-α was actually increased in OAT of obese subjects. Adipose tissue TNF-α mRNA abundance was

![Graph](image-url)
increased 2-fold higher in obese than in lean subjects [relative expression: 1.99 ± 0.31 vs. 1.00 ± 0.28, n = 13 (obese) and 8 (lean); P < 0.05]. We next examined which subcellular fraction was the main contributor to TNF-α production (Fig. 2A, A and B). Adipose tissue was fractionated into isolated adipocytes and SVCs, which were cultured independently for 24 h. TNF-α secretion by both fractions was higher in obesity. TNF-α secretion by SVCs was also higher than that by adipocytes independently of the BMI. Qualitatively similar changes were observed for TNF-α gene expression (Fig. 2B).

We also compared the distribution of the two TNF-α receptors in this model. Whereas gene expression of both TNFR1 and TNFR2 was enhanced in SVCs of obese subjects compared with lean ones, only TNFR2 was increased in obese adipocytes (Fig. 2, C and D).

Mature adipocytes freshly isolated from omental fat of lean or obese subjects were used in all subsequent culture experiments.

**Effects of SVC-conditioned media on adipokine expression by mature adipocytes**

Because TNF-α secretion is augmented in obesity and higher in SVCs, we examined whether media conditioned by SVCs mimicked the effects of TNF-α. Mature adipocytes isolated from lean and obese subjects were cultured in the presence of SVC-conditioned media. These media had been previously collected after culturing independently SVCs from lean or obese subjects; before use (i.e., after mixing with fresh medium), their final concentrations of TNF-α amounted to 0.08 ± 0.02 and to 0.22 ± 0.05 pg/ml when originally obtained from lean (n = 8) or obese (n = 13) individuals, respectively (P < 0.01). Conditioned media (from either lean or obese subjects) barely affected mRNA expression of the investigated adipokines in mature adipocytes of lean subjects. By contrast, in obese adipocytes, media conditioned by obese SVC induced an approximately 2-fold rise in gene expression of all the investigated adipokines (except for TPO whose expression was halved) (Fig. 3A). Thus, the effects of SVC-conditioned media on mature adipocytes reproduced those of TNF-α on *in vitro* differentiated adipocytes, and mature adipocytes of obese subjects were more responsive to these conditioned media.

Because SVCs release numerous regulatory factors, we examined whether the changes induced by SVC-conditioned media were specifically due to TNF-α. Adipocytes of additional obese subjects were cultured in media conditioned by obese SVCs, with or without anti-TNF-α antibody (Fig. 3B). The effects of obese SVC-conditioned media on adipokine mRNAs were largely prevented by immunoneutralization of TNF-α activity.

Eventually we directly tested TNF-α on mature adipocytes and compared the responses between lean and obese subjects (Fig. 4). Apart from for TPO, TNF-α did not cause any significant change of adipokine mRNAs in adipocytes of lean subjects even at the highest concentration used. By contrast, in obese subjects, significant increases were already detected at the lowest concentration (0.1 ng/ml) and mostly occurred in a dose-dependent fashion. These data indicate that adipocytes of obese subjects are hyperresponsive to TNF-α.

**Mechanisms underlying the hyperresponsiveness of obese adipocytes to TNF-α**

We studied TNF-α receptor and postreceptor signaling. We first investigated whether up-regulation of TNFR2 could mediate the hyperresponsiveness of obese adipocytes. However, only TNFR1 (but not TNFR2, data not shown) immunoneutralization did actually prevent most changes of adipokine mRNAs induced by TNF-α in adipocytes of obese subjects (Table 2).

We next explored which events downstream to TNFR1 could be involved. We studied the effects of NF-κB (BAY11-7085) and JNK inhibitors (SP600125) on TNF-α-induced changes of adipokine expression in mature obese adipocytes (Table 2). Blockade of NF-κB, but not the JNK pathway, did largely prevent alterations of adipokine mRNAs caused by TNF-α.

Because activation of NF-κB pathway may underlie the hyperresponsiveness of obese adipocytes to TNF-α, we directly measured NF-κB (p65) DNA binding activity in mature adipocytes of lean and obese subjects, in the absence or in the presence of TNF-α. TNF-α induced a slight but significant increase of NF-κB activity (∼16%) in lean adipocytes (Fig. 5A). In obese adipocytes, both basal and TNF-α-stimulated values of NF-κB activity were about 75–95% higher than in lean adipocytes (Fig. 5A). When the increment of NF-κB activity induced by TNF-α (Δ) was expressed as percentage of the respective basal values within each group of lean and obese subjects, there was still a
2-fold difference between the two groups (Fig. 5B). This suggests that the hyperresponsiveness of obese adipocytes to TNF-α could be mediated by an increased responsiveness of NF-κB activity.

There were positive relationships between values of NF-κB activity and mRNA abundance of each investigated adipokine in TNF-α-treated conditions [Pearson’s correlation coefficients $r$ ranging between 0.59 and 0.74, $P < 0.05$ or less; $n = 13$ (6 lean, 7 obese) subjects]. Adipocyte size was related to the BMI ($r = 0.65$, $P < 0.05$; $n = 13$), and there were positive relationships between NF-κB activity and the BMI or adipocyte size (Fig. 5, C and D). Eventually, Δ NF-κB activity was also positively correlated with the HOMA-IR (Fig. 5E). There was no correlation between any NF-κB parameters and other clinical or laboratory measurements (systolic or diastolic blood pressure, total or low-density lipoprotein or low-density lipoprotein cholesterol, triglycerides, adiponectin, or leptin). Multiple regression analysis showed that BMI or adipocyte size was a significant independent determinant of adipocyte NF-κB activity explaining up to 44% (BMI) and 49% (cell size) of its variance. Insulin resistance was also a significant predictive factor for Δ NF-κB activity, explaining up to 64% of its variance.

Discussion

TNF-α was overexpressed in omental adipose tissue of obese subjects, as originally described in sc fat (16, 17) and was more abundantly produced by SVCs than adipocytes, in agreement with pooled data from both adipose depots of obese subjects (18). Strikingly, TNF-α secretion by obese SVCs was 20-fold larger than that by lean adipocytes. This has to be ascribed to the altered cellular composition of the SV fraction in obesity, which is characterized by an increased number of macrophages and endothelial cells, two cell types that largely contribute to the production of proinflammatory factors (5, 19).

Exposure of mature adipocytes to SVC-conditioned media, especially from obese subjects, induced a pattern of adipokine gene expression similar to that observed after TNF-α treatment. One should expect corresponding changes of adipokine mRNAs and protein secretion, as previously shown (see Fig. 1). TNF-α immunoneutralization of these SVC-conditioned media specifically prevented adipokine mRNA changes, suggesting that TNF-α is a major SVC mediator of adipokine dysregulation in adipocytes. Coculture of murine 3T3-L1 adipocytes and macrophage cell line did result in marked up-regulation of proinflammatory adipokines, which was inhibited by a neutralizing antibody to TNF-α (13). By using a more physiologically relevant source of proinflammatory cytokines (i.e., SVCs), our data broaden the concept that, among all the numerous factors released by SVCs, TNF-α plays a crucial paracrine role in worsening adipocyte inflammation in humans. Hence, based on our original observations, TNF-α appears to be a potent and proximal molecule in the hierarchy of cytokines’ and chemokines’ networks of human adipose tissue.
Alteredations of adipokine gene expression induced by either SVC-conditioned media or direct exposure to TNF-α were amplified in adipocytes of obese subjects compared with lean ones. This hyperresponsiveness of obese adipocytes to an inflammatory stimulus is a novel finding that sharply contrasts with the marked resistance to insulin, another hallmark of the metabolic syndrome. This hyperresponsiveness was already triggered by the lowest concentration of TNF-α used (0.1 ng/ml, Fig. 5), which was within the same range as that measured in SVC-conditioned media. Adipocyte size has recently been shown to be an important determinant of dysregulated adipokine expression and secretion, with the hypertrophic adipocytes shifting their immune balance toward the production of proinflammatory molecules (3–5). We extend these data, which had been previously obtained in the basal state, to adipocytes acutely challenged by a proinflammatory stimulus. This paradigm may perhaps explain why there was no difference in TNF-α response between adipocytes differentiated in vitro from stromal precursors of obese vs. nonobese donors as a similar degree of fat cell hypertrophy was achieved in both situations (our own data).

The primary molecular mechanisms whereby TNF-α triggers an excessive inflammatory response in hypertrophied adipocytes are still unsettled. We explored the signaling pathways at the receptors’ and postreceptors’ levels. In agreement with data obtained in whole adipose tissue (20, 21), TNFR2, but not TNFR1, was increased in adipocytes isolated from obese subjects. TNFR2 has been proposed to mediate some effects of TNF-α in chronic inflammatory states such as obesity in both rodents (22) and humans (20, 23, 24). However, our immunoneutralization experiments ruled out a role for TNFR2 in overstimulating adipokine production and clearly established that this effect of TNF-α was mediated by TNFR1, in line with most effects of TNF-α on adipocyte tissue (25). Dissection of downstream signals indicates a pivotal role for the transcriptional factor NF-κB in mediating TNF-α-induced adipokine production, in line with other reports (26–28). Yet the activity and the functional sig-
Here we compared NF-κB and TNF-α expression in adipocytes of lean and obese subjects during adipocyte differentiation and hypertrophy (29). In our study, NF-κB and TNF-α activities were higher in adipocytes of obese subjects than in those of lean subjects. Accordingly, an increase in constitutive NF-κB activity has been reported in 3T3-L1 cell line during adipocyte differentiation and hypertrophy (29). In our study, NF-κB activity did correlate with the expression of each adipokine and adipocyte size, reinforcing the concept that adipocyte hypertrophy plays a crucial role in triggering inflammation. Eventually the response of NF-κB activity to TNF-α also correlated with insulin resistance (IR). Mature adipocytes of lean and obese subjects were cultured with or without 10 ng/ml TNF-α for 4 h. NF-κB (p65) DNA-binding activity was quantified by ELISA in whole-cell protein extracts. Measurements were expressed as OD units and normalized to levels of a cellular DNA control. Results are the mean ± SEM for adipocytes of six lean and seven obese subjects. *, P < 0.05; **, P < 0.01 for the effect of TNF-α vs. respective untreated conditions; +, P < 0.05 for the effect of obesity vs. respective lean conditions. Lower panels, Relationships between adipocyte NF-κB activity and BMI, adipocyte size, or HOMA-IR indices. Stimulated values of NF-κB activity (percent of untreated lean data) are shown in C and D, while similar correlations were obtained with the basal values (not illustrated). Δ NF-κB activity values are presented as raw data in E. Correlation analysis was performed in the six lean (A) and seven obese subjects (BC) studied together. Dotted lines correspond to the 95% confidence interval for r.

FIG. 5. Effects of TNF-α on NF-κB DNA binding activity in mature adipocytes and relationships with BMI, adipocyte size, and insulin resistance (IR). Mature adipocytes of lean and obese subjects were cultured with or without 10 ng/ml TNF-α for 4 h. NF-κB (p65) DNA-binding activity was quantified by ELISA in whole-cell protein extracts. Measurements were expressed as OD units and normalized on a cellular basis (per microgram adipocyte DNA). Upper panels, Data are presented as percentages of values in untreated lean adipocytes (A) or increments induced by TNF-α (stimulated minus basal values) (B, in percent of the respective basal values in each group). Results are the mean ± SEM for adipocytes of six lean and seven obese subjects. *, P < 0.05; **, P < 0.01 for the effect of TNF-α vs. respective untreated conditions; +, P < 0.05 for the effect of obesity vs. respective lean conditions. Lower panels, Relationships between adipocyte NF-κB activity and BMI, adipocyte size, or HOMA-IR indices. Stimulated values of NF-κB activity (percent of untreated lean data) are shown in C and D, but similar correlations were obtained with the basal values (not illustrated). Δ NF-κB activity values are presented as raw data in E. Correlation analysis was performed in the six lean (A) and seven obese subjects (BC) studied together. Dotted lines correspond to the 95% confidence interval for r.

clearly shown a role for TNF-α in systemic insulin sensitivity in humans (34–36).

In obesity, the hyperresponsiveness of hypertrophied adipocytes to TNF-α coupled to an oversecretion of this cytokine by SVCs appears to be teleologically meaningful. This amplified paracrine cross talk involving TNF-α may be viewed as an ultimate attempt to control adiposity. Enhanced TNF-α (action/production) aims at limiting further weight gain through lipolysis and insulin resistance, impaired preadipocyte differentiation, and increased adipocyte apoptosis (37). However, these effects of TNF-α occur at the expense of worsening insulin resistance and inflammation. This insulin-resistant and inflammatory state may further be amplified by the overproduction of the investigated adipokines (5), thereby linking obesity to its comorbidities.

In conclusion, TNF-α that, among all the numerous factors secreted by SVCs, is a crucial determinant of adipokine dysregulation in obesity acts on enlarged adipocytes, which are hyperresponsive to this inflammatory triggering signal. This in vitro adipocyte hyperresponsiveness results in adipokine overproduction through TNFR1 and hyperactivation of NF-κB. This amplified paracrine cross talk between adipocytes and SVCs in human obesity ultimately leads to worsening of the inflammatory state and the metabolic syndrome.

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