**In vitro** evidence that hyperglycemia stimulates tumor necrosis factor-α release in obese women with polycystic ovary syndrome

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Abstract

Women with polycystic ovary syndrome (PCOS) are often insulin resistant and have chronic low-level inflammation. The purpose of this study was to determine the effects of hyperglycemia in vitro on tumor necrosis factor (TNF)-α release from mononuclear cells (MNC) in PCOS. Twelve reproductive-age women with PCOS (six lean, six obese) and 12 age-matched controls (six lean, six obese) were studied. Insulin sensitivity (IS_HOMA) was estimated from fasting levels of glucose and insulin and percent trunkal fat was determined by dual energy absorptiometry (DEXA). TNFα release was measured from MNC cultured under euglycemic and hyperglycemic conditions. IS_HOMA was higher in obese women with PCOS than in lean women with PCOS (student’s t-test; 73.7 ± 14.8 vs 43.1 ± 8.6, P<0.05), but similar to that of obese controls. IS_HOMA was positively correlated with percent trunkal fat (r=0.57, P<0.04). Obese women with PCOS exhibited an increase in the percent change in TNFα release from MNC in response to hyperglycemia compared with obese controls (10 mM, 649 ± 208% vs 133 ± 30%, P<0.003; 15 mM, 799 ± 347% vs 183 ± 59%, P<0.04). The TNFα response directly correlated with percent trunkal fat (r=0.45, P<0.03) and IS_HOMA (r=0.40, P<0.05) for the combined groups, and with plasma testosterone (r=0.60, P<0.05) for women with PCOS. MNC of obese women with PCOS exhibit an increased TNFα response to in vitro physiologic hyperglycemia. MNC-derived TNFα release may contribute to insulin resistance and hyperandrogenism, particularly when the combination of PCOS and increased adiposity is present.


Introduction

Polycystic ovary syndrome (PCOS) is one of the most common female endocrinopathies, affecting 4–10% of reproductive-age women (Knochenhauer et al. 1998, Dunaif 1999). The disorder is characterized by hyperandrogenism, chronic oligo- or anovulation, and polycystic ovaries, with two out of these three findings required to diagnose PCOS (Rotterdam Group 2004a, 2004b). As many as 70% of women with PCOS exhibit insulin resistance, with the compensatory hyperinsulinemia considered to be the cause of the hyperandrogenism (Burghen et al. 1980, Nestler et al. 1998, Goodarzi & Korenman 2002, Rotterdam Group 2004a, 2004b). In addition, women with PCOS are often obese, a condition strongly associated with insulin resistance and hyperglycemia (Kolterman et al. 1980, Ciaraldi et al. 1981).

Hyperglycemia can contribute to the development of insulin resistance and impaired insulin secretion in a phenomenon known as ‘glucose toxicity’ (Rossetti et al. 1990, Yki-Jarvinen 1992). It is recognized that these effects may be the exaggeration of normal regulatory responses to increases in circulating glucose. We have shown that in PCOS, hyperglycemia causes an increase in reactive oxygen species (ROS) generation from peripheral blood mononuclear cells (MNC) (González et al. 2006). ROS-induced oxidative stress is a known activator of nuclear factor κB (NFκB), a proinflammatory transcription factor that promotes tumor necrosis factor-α (TNFα) gene transcription (Barnes & Karin 1997, Mohanty et al. 2000, Evans et al. 2002). TNFα is an established mediator of insulin resistance (Hotamisligil et al. 1994). Thus, increased TNFα release from MNC in response to hyperglycemia may be an underlying mechanism for insulin resistance in PCOS.

In vitro studies have shown that TNFα can truncate insulin receptor signaling in all insulin-sensitive tissues (Feinstein et al. 1993, Hotamisligil et al. 1994, Del Aguila et al. 1999). In obesity-related diabetic syndromes, TNFα is overexpressed in adipose tissue and causes increased serine phosphorylation of insulin receptor substrate-1 (IRS-1) (Hotamisligil et al. 1993, 1995, Rui et al. 2001). This leads to decreased expression of GLUT 4, the insulin-sensitive glucose transport protein (Stephens &
Insulin resistance in PCOS is also a post-receptor defect, and increased serine phosphorylation is implicated as the cause of decreased insulin-stimulated IRS-1 activation and decreased GLUT 4 expression (Rosenbaum et al. 1993, Dunai et al. 2001, Li et al. 2002, Corbould et al. 2005). Thus, the ability of TNFα to stimulate increased serine phosphorylation makes it an ideal candidate for initiating these molecular events in PCOS.

We have previously reported that circulating levels of TNFα are elevated in PCOS (González et al. 1999). A likely source of excess circulating TNFα in obese women with PCOS is adipose tissue, but the source remains unknown in lean women with the disorder. MNC are known to migrate into adipose tissue to activate adipocyte TNFα production (Weisberg et al. 2003, Wellen & Hotamisligil 2003). It is now clear that the major source of TNFα in adipose tissue of the obese is MNC-derived macrophages present in the stromal–vascular compartment (Weisberg et al. 2003, Xu et al. 2003, Fain et al. 2004a, 2004b). Thus, MNC may be an additional source of excess circulating TNFα in PCOS.

In the present study, we evaluated an in vitro model of hyperglycemia to determine the effect of direct exposure to hyperglycemia on TNFα release from MNC of women with PCOS. We hypothesized that TNFα release from MNC is increased in women with PCOS compared with weight-matched controls in response to hyperglycemic conditions, and that there is a relationship between measures of adiposity and MNC-derived TNFα release.

Materials and Methods

Subjects

Twelve women with PCOS (six lean and six obese) aged 21–34 years and 12 weight-matched control subjects (six lean and six obese) aged 20–38 years volunteered to participate in the study. The women with PCOS were diagnosed on the basis of oligoamenorrhea and hyper-androgenemia after excluding nonclassic congenital adrenal hyperplasia, Cushing’s syndrome, hyperprolactinemia and thyroid disease. Polycystic ovaries were present on ultrasound in all subjects with PCOS. All control subjects were ovulatory as evidenced by regular menses and a luteal phase serum progesterone level greater than 5 ng/ml. All control subjects exhibited normal circulating androgen levels and the absence of polycystic ovaries on ultrasound.

All subjects were screened for diabetes or inflammatory illnesses, and none were taking medications that affect carbohydrate metabolism or immune function for at least 6 weeks prior to study participation. None of the subjects were involved in any regular exercise program for at least 6 months before the time of testing. All of the subjects provided written, informed consent in accordance with the Case Western Reserve University and Metro-Health Medical Center guidelines for the protection of human subjects.

Study design

All study subjects underwent the oral glucose tolerance test (OGTT) on days 5 and 8 after the onset of menses. Before the OGTT, they were provided with a healthy diet consisting of 50% carbohydrate, 35% fat and 15% protein for 3 consecutive days (days 1–3) before the test. The test was performed on the morning of day 4 after an overnight fast of ~12 h. All subjects also underwent body composition assessment on the same day the OGTT was performed.

Oral glucose tolerance test (OGTT)

Fasting baseline blood samples (5 ml each) were drawn for glucose and insulin determination. A 75 g glucose beverage was subsequently ingested over 10 min. Blood samples (5 ml each) were again drawn for glucose and insulin determination 2 h after glucose ingestion. Upon completion of the test, subjects were fed a high-carbohydrate snack. Plasma glucose concentrations were assayed immediately from the blood samples collected. Additional plasma was isolated from the fasting blood samples and stored at −70°C until assayed for C-reactive protein (CRP) and TNFα. Glucose tolerance was assessed by the WHO criteria with normal glucose tolerance defined as a 2-h glucose-stimulated value less than 140 mg/dl, impaired glucose tolerance as a 2-h value of 140–199 mg/dl, and type 2 diabetes mellitus defined as a 2-h value of 200 mg/dl or greater (Modan et al. 1989). Insulin sensitivity was estimated by IS_HOMA by the following formula (Matthews et al. 1985): fasting glucose × fasting insulin/22.5.

Body composition assessment

Height without shoes was measured to the nearest 1·0 cm. Body weight was measured to the nearest 0·1 kg. Waist circumference was measured at the level of the umbilicus and used to estimate abdominal adiposity (Kohrt et al. 1993). In addition, all subjects underwent dual energy absorptiometry (DEXA) to determine percent total body fat and percent truncal fat with the QDR 4500 Elite model scanner (Hologic, Waltham, MA, USA). Truncal fat content was defined as the area between the dome of the diaphragm (cephalad limit) and the top of the greater trochanter (caudal limit) (Taylor et al. 1998).

Analytic methods

MNC isolation and culture were performed on a 20 ml fasting blood sample drawn before ingestion of the
glucose beverage during the OGTT. The cells were isolated by Histopaque-1077 density gradient centrifuga-
tion (Boyam 1968), washed two times in pyrogen-free
saline, re-suspended in RPMI (0·3 mg/ml l-glutamine,
100 U/ml penicillin and 100 μg/ml streptomycin) with
serum substitute TCH, and seeded in coated culture plates
(2·510⁶ cells/ml). The culture medium was supplemented
with d-glucose at varying concentrations to mimic a
euglycemic (5 mM) or hyperglycemic (10 or 15 mM)
environment. The cells were incubated (humidified, 5%
CO₂, 37° C) for 24 h. Cell supernatants were subse-
sequently collected (10 000 g for 2 min) and stored at
−70° C until analysis. Plasma glucose concentrations were
measured by the glucose oxidase method (YSI, Yellow
Springs, OH, USA), while plasma insulin concentra-
tions were measured by double-antibody RIA (Linco
Research, St Charles, MO, USA). Plasma CRP concen-
trations were measured by high-sensitivity ELISA (Alpha
Diagnostics International, San Antonio, TX, USA).
TNFα concentrations were also measured by ELISA
(BioSource International, Camarillo, CA, USA). All
samples from each subject were measured in duplicate in
the same assay. The interassay and intra-assay coefficients
of variation for all assays were 7% and 12% respectively.

Results
Age and height were similar among groups (Table 1).
Weight, body-mass index (BMI), percent total body fat
and waist circumference were significantly (P<0·01)
greater in obese subjects than in those who were lean
whether or not they had PCOS, but were similar when
women with PCOS were compared with weight-
matched controls. The obese also exhibited significantly
(P<0·01) greater percent truncal fat than lean subjects
whether or not they had PCOS. However, percent
truncal fat was significantly (P<0·02) greater in lean
women with PCOS than in lean controls.

Circulating levels of LH, testosterone and andro-
stenedione were significantly (P<0·05) higher in women
with PCOS than in control subjects independently of body
weight (Table 2). Circulating DHEA-S levels were modestly
higher in women with PCOS than in weight-matched
controls. Levels of glucose while fasting and 2 h after glucose
ingestion were similar in women with PCOS to those of
lean controls. The interassay and intra-assay coefficients
of variation for all assays were 7% and 12% respectively.

Statistics
The StatView statistical package (SAS Institute, Cary, NC,
USA) was used for data analysis. The difference in values
under either hyperglycemic condition (10 or 15 mM) and
the euglycemic baseline (5 mM) for primary dependent
variables, such as TNFα release from MNC, was calcu-
lated to represent the incremental change. Descriptive
data and the incremental change of variables were com-
pared between groups by unpaired Student’s t-test or
ANOVA for multiple-group comparisons followed by
post hoc analysis. Differences between the incremental
change of variables within groups were analyzed by paired
Student’s t-test. Alterations in TNFα release from MNC
within groups were expressed as the percent change
between either hyperglycemic condition (10 or 15 mM)
and the euglycemic condition (5 mM, 100% baseline).
Differences in the MNC-derived TNFα response among
the different glycemic conditions within groups were
analyzed by repeated-measures ANOVA. Regression
analyses used the Pearson (r) correlation for parametric
data and the Spearman rank order (p) correlation for
nonparametric data. All values are expressed as means ±
S.E.M. An α-level of 0·05 was used to determine
statistical significance.

Table 1 Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control Lean (n=6)</th>
<th>Obese (n=6)</th>
<th>PCOS Lean (n=6)</th>
<th>Obese (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33 ± 2</td>
<td>30 ± 3</td>
<td>26 ± 2</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165·6 ± 1·0</td>
<td>163·1 ± 3·6</td>
<td>165·4 ± 3·9</td>
<td>165·7 ± 2·4</td>
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<tr>
<td>Body weight (kg)</td>
<td>59·1 ± 2·2</td>
<td>91·9 ± 4·7b</td>
<td>63·9 ± 3·5</td>
<td>96·6 ± 6·0c,d</td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>21·6 ± 0·8</td>
<td>34·5 ± 1·2a</td>
<td>23·3 ± 0·5</td>
<td>35·2 ± 1·6c,d</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>27·1 ± 1·8</td>
<td>43·8 ± 0·4ab</td>
<td>30·7 ± 1·7</td>
<td>44·2 ± 1·0cd</td>
</tr>
<tr>
<td>Truncal fat (%)</td>
<td>22·8 ± 2·4</td>
<td>43·2 ± 0·6ab</td>
<td>29·7 ± 2·6</td>
<td>45·2 ± 0·9cd</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>71·3 ± 2·0</td>
<td>101·1 ± 3·4ab</td>
<td>78·0 ± 2·7</td>
<td>96·2 ± 8·0c,d</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.E. aObese controls vs lean controls, P<0·0003; bObese controls vs lean PCOS, P<0·002; cObese PCOS vs lean controls, P<0·01; dLean PCOS vs lean controls, P<0·02.
were modestly higher, and plasma CRP levels were significantly higher ($P<0.05$) in the obese whether or not they had PCOS (Table 2). CRP levels were also modestly elevated in lean women with PCOS compared with lean controls.

$\text{IS}_{\text{HOMA}}$ was significantly ($P<0.05$) higher in obese women with PCOS than in lean controls and lean women with PCOS (Table 2). Lean women with PCOS exhibited an $\text{IS}_{\text{HOMA}}$ that was similar to that of obese controls and lean controls. $\text{IS}_{\text{HOMA}}$ was positively correlated with BMI ($r=0.58$, $P=0.004$), percent body fat ($r=0.49$, $P<0.02$) and percent truncal fat ($r=0.57$, $P<0.004$) for the combined groups (data not shown).

$\text{TNF}_\alpha$ release from MNC under euglycemic conditions (5 mM) was similar in women with PCOS compared with controls (Table 3). After exposure of MNC to hyperglycemia at the 10 mM glucose concentration, the incremental change in $\text{TNF}_\alpha$ release with women with PCOS increased significantly ($P<0.03$) compared with that of controls, which slightly declined (7.0 ± 3.3 vs. −2.9 ± 2.2). When subjects were grouped by body mass, the incremental change in $\text{TNF}_\alpha$ release from MNC exposed to 10 mM glucose increased significantly in obese women with PCOS compared with either lean controls ($P<0.005$) or obese controls ($P<0.05$). In contrast, the incremental change in $\text{TNF}_\alpha$ release from MNC of lean women with PCOS was similar to lean controls after the 10 mM glucose exposure. There were no significant differences in the $\text{TNF}_\alpha$ response of women with PCOS compared with control subjects regardless of body weight after exposure of MNC to 15 mM glucose.

The percent change in $\text{TNF}_\alpha$ release from MNC in control subjects remained unchanged under hyperglycemic conditions (10 mM, 103 ± 20%; 15 mM, 136 ± 33%) compared with the euglycemic condition (5 mM, 100%). In contrast, the women with PCOS exhibited a significant ($P<0.02$) progressive increase in the percent change in $\text{TNF}_\alpha$ release from MNC under hyperglycemic conditions compared with the euglycemic condition. The MNC-derived $\text{TNF}_\alpha$ response to hyperglycemia was significantly greater in women with PCOS (10 mM, 410 ± 124%, $P<0.03$; 15 mM, 541 ± 200%, $P<0.05$) than in controls. When subjects were grouped by body mass, lean controls exhibited no difference in the percent change in $\text{TNF}_\alpha$ release from MNC under hyperglycemic conditions compared with the euglycemic condition (Fig. 1). Obese controls and lean women with PCOS exhibited a modest increase in the percent change in

### Table 2 Plasma hormone, glucose, insulin and C-reactive protein (CRP) levels

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PCOS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Lean (n=6)</td>
<td>Obese (n=6)</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>3·2 ± 0·4</td>
<td>2·6 ± 0·3</td>
</tr>
<tr>
<td>Testosterone (ng/dl)</td>
<td>42·8 ± 5·8</td>
<td>32·7 ± 6·2</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>1·8 ± 0·2</td>
<td>2·0 ± 0·1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHEA-S (μg/dl)</td>
<td>167 ± 20</td>
<td>205 ± 29</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>85·3 ± 2·0</td>
<td>84·3 ± 3·2</td>
</tr>
<tr>
<td>2-h glucose (mg/dl)</td>
<td>99·0 ± 11·8</td>
<td>121·8 ± 6·0</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>8·5 ± 1·5</td>
<td>137 ± 2·5</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>219 ± 9·2</td>
<td>6935 ± 139&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>$\text{IS}_{\text{HOMA}}$</td>
<td>32·1 ± 4·5</td>
<td>52·8 ± 10·7</td>
</tr>
</tbody>
</table>

Values are expressed as means ± s.e. *Lean PCOS vs lean controls, P<0·005*; *Lean PCOS vs obese controls, P<0·003; *Lean PCOS vs obese PCOS, P<0·05*; *Obese PCOS vs lean controls, P<0·02; *Obese PCOS vs lean controls, P<0·05; *Obese controls vs lean controls, P<0·0005.

### Table 3 $\text{TNF}_\alpha$ release from mononuclear cells (MNC) under euglycemic and hyperglycemic conditions in vitro

<table>
<thead>
<tr>
<th>MNC $\text{TNF}_\alpha$ (pg/ml)</th>
<th>5 mM</th>
<th>10 mM</th>
<th>Δ</th>
<th>15 mM</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean controls (n=6)</td>
<td>9·4 ± 4·1</td>
<td>3·6 ± 0·8</td>
<td>−5·8 ± 4·0</td>
<td>6·4 ± 1·8</td>
<td>−3·0 ± 2·7</td>
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<tr>
<td>Obese controls (n=6)</td>
<td>4·5 ± 1·4</td>
<td>4·5 ± 1·5</td>
<td>0·02 ± 1·6</td>
<td>8·5 ± 5·3</td>
<td>8·5 ± 5·3</td>
</tr>
<tr>
<td>Lean PCOS (n=6)</td>
<td>3·0 ± 0·4</td>
<td>5·4 ± 1·8</td>
<td>2·4 ± 1·6</td>
<td>7·9 ± 4·5</td>
<td>4·9 ± 4·5</td>
</tr>
<tr>
<td>Obese PCOS (n=6)</td>
<td>3·0 ± 0·7</td>
<td>14·5 ± 6·1</td>
<td>11·6 ± 6·0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>21·9 ± 13·6</td>
<td>19·0 ± 13·3</td>
</tr>
</tbody>
</table>

Values are expressed as means ± s.e. Δ: calculated differences between $\text{TNF}_\alpha$ concentrations under either hyperglycemic condition (10 or 15 mM) and euglycemic baseline (5 mM).

*Obese PCOS vs lean controls, P<0·005; *Obese PCOS vs obese controls, P<0·05.
TNFα release from MNC in response to hyperglycemia compared with lean controls. However, there was a significant increase in the MNC-derived TNFα response to hyperglycemia in obese women with PCOS (10 mM, P < 0.003; 15 mM, P < 0.04) compared with either control group.

After exposure of MNC to hyperglycemia at the 10 mM glucose concentration, the percent change in TNFα release from MNC was positively correlated with BMI, percent truncal fat and IS_HOMA for the combined groups, and with BMI for women with PCOS (Table 4). The percent change in TNFα release from MNC was also positively correlated with androstenedione for the combined groups after exposure to both 10 and 15 mM glucose, and for women with PCOS after exposure to 15 mM glucose. The percent change in TNFα release from MNC and testosterone were positively correlated for women with PCOS after exposure to 15 mM glucose, and negatively correlated for controls after exposure to both 10 and 15 mM glucose.

**Discussion**

Our data clearly show that in vitro exposure to hyperglycemia causes an increase in TNFα release from MNC of obese women with PCOS. The in vitro conditions demonstrate that the enhanced TNFα response is directly related to hyperglycemia, and occurs at a glucose concentration similar to the postprandial state (10 mM) or higher (15 mM). The TNFα response is directly related to the degree of insulin resistance and to the levels of androgens. Since TNFα is a proinflammatory cytokine, these findings provide further support for the role of inflammation in the development of insulin resistance and hyperandrogenism in PCOS. TNFα, in particular, may contribute to the decline in insulin action in obese women with PCOS. Furthermore, the association of TNFα with BMI, percent total body fat and percent truncal fat suggests that the combination of PCOS and increased adiposity may be a key determinant of the MNC-derived TNFα response.
Hyperglycemia within the physiologic range does not result in increased TNFα release from MNC in normal circumstances. Lean controls in the present study exhibited no change in MNC-derived TNFα release in response to either hyperglycemic culture condition. This is in contrast to a previous report of increases in TNFα release from MNC of normal individuals exposed to hyperglycemia in vivo (Morohoshi et al. 1996). In this study, however, the glucose concentration required to achieve this response was above the physiologic range. Since TNFα is a known mediator of insulin resistance (Hotamisligil et al. 1994, 1995, Del Aguila et al. 1999), the lack of increase in TNFα release from MNC may be a physiologic benefit in the presence of hyperglycemia. Thus, facilitation of glucose disposal in lean controls may be due to the control of TNFα release to optimize insulin signaling in the postprandial state.

In contrast, the MNC of obese women with PCOS have increased sensitivity to hyperglycemic conditions in the physiologic range. Obese women with PCOS are in a proinflammatory state, as shown by the elevations of plasma CRP observed in this group that are consistent with previous reports (Kelly et al. 1996, Bastard et al. 1999, Yudkin et al. 1999). MNC-derived TNFα release increased in response to either hyperglycemic culture condition in obese women with PCOS compared with obese controls. Oral intake of glucose, lipid and protein has been noted to elicit similar proinflammatory responses in vivo (Mohanty et al. 2000, 2002, Aljada et al. 2004). It is possible that in obese women with PCOS, feeding results in increased TNFα release from MNC in the postprandial state to promote the insulin resistance observed in these individuals. This concept is supported by the positive correlation between the TNFα response and IS_HOMA.

Hyperglycemia and TNFα release in PCOS.

Previous reports of a reduction in oxidative stress and inflammatory mediators after caloric restriction in the obese, and after a 2-day fast in normal subjects provide further corroboration (Dandona et al. 1998, 2001a, 2001b).

Our data suggest a link between adiposity and MNC-derived TNFα release in PCOS. There was a direct relationship between the change in TNFα release from MNC under hyperglycemic culture conditions and abdominal adiposity. It is possible that the inflamed adipose tissue in the abdominal region of obese women with PCOS perpetuates the increased sensitivity of MNC to hyperglycemia manifested by the increased TNFα release observed in culture. Our data also demonstrate a direct relationship between the degree of insulin resistance by IS_HOMA and abdominal adiposity. These findings are consistent with previous observations in young adults demonstrating that changes in insulin sensitivity are a function of abdominal adiposity (Krikketos et al. 2004, Linne 2004). Thus, increased TNFα release from MNC may promote the insulin resistance observed in obese women with PCOS.

The MNC of obese controls and lean women with PCOS exhibited only modest increases in TNFα release in response to hyperglycemia. Both of these groups are in a proinflammatory state, as evidenced by the elevated CRP concentrations in accordance with previous observations (Kelly et al. 1996, Boulman et al. 2004). The lack of statistical significance in the increases in TNFα release in either of these groups and the elevated CRP concentrations in lean women with PCOS may be due to the small sample size. Nevertheless, there is a stepwise increasing trend in the TNFα response, with progressively higher
hyperglycemic conditions when study subjects are grouped by body weight. It is possible that the increased abdominal adiposity observed in lean women with PCOS also perpetuates increases in hyperglycemia-induced TNFα release from MNC to promote insulin resistance in this group. Nevertheless, the presence of PCOS in combination with a greater amount of adiposity may explain the higher TNFα response and greater degree of insulin resistance evident in obese women with PCOS.

In PCOS, TNFα release from MNC in response to hyperglycemia may be capable of directly stimulating hyperandrogenism. This is suggested by the direct correlation of the TNFα response with plasma levels of testosterone and androstenedione in women with PCOS. We have also demonstrated direct correlations of ROS generation (González et al. 2006) and activated NFκB (unpublished data) with these androgen levels. Infiltration of the ovary by MNC-derived macrophages has been previously reported (Best et al. 1996). Ovarian steroidogenic enzymes responsible for androgen production are stimulated by oxidative stress and inhibited by antioxidants, such as statins, in vitro (Piotrowski et al. 2005, Rzepczynska et al. 2005). Circulating androgen levels decline in women with PCOS in response to statin therapy in vitro (Banaszewska et al. 2005). TNFα stimulates proliferation of androgen-producing theca cells (Spazynsky et al. 1999). Thus, it is attractive to consider that increased TNFα release from glucose-activated MNC recruited into the polycystic ovary may be the result of a local inflammatory response that stimulates ovarian androgen production in women with PCOS.

In conclusion, MNC of obese women with PCOS exhibit increased TNFα response when directly exposed to hyperglycemic conditions in vitro in the physiologic range. Our findings suggest that the increased abdominal adiposity in women with PCOS promotes a proinflammatory state, especially in those who are obese. The associations of the TNFα response with measures of adiposity and androgen levels suggest that MNC-derived TNFα release contributes to insulin resistance and hyperandrogenism, particularly when the combination of PCOS and increased adiposity is present.

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