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Altered tumor necrosis factor α release from mononuclear cells of obese reproductive-age women during hyperglycemia

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Abstract

The aim of the study was to determine whether lipopolysaccharide (LPS)-stimulated tumor necrosis factor α (TNF-α) release from mononuclear cells (MNCs) is altered in obese reproductive-age women in response to hyperglycemia. Six obese and 8 age-matched normal-weight women (18-40 years) underwent a 2-hour 75-g oral glucose tolerance test. Tumor necrosis factor α release was measured from MNCs cultured in the presence of LPS after isolation from blood samples drawn fasting and 2 hours after glucose ingestion. Insulin resistance was derived by homeostasis model assessment of insulin resistance. Total body fat (%) and truncal fat (%) were determined by dual-energy absorptiometry. Obese women had a higher ($P < .03$) body mass index (34.1 ± 1.1 vs 21.9 ± 0.8 kg/m^2), percentage of total body fat (42.4% ± 1.3% vs 28.7% ± 1.8%), and percentage of truncal fat (42.1% ± 1.2% vs 24.7% ± 2.2%). Homeostasis model assessment of insulin resistance was greater in the obese group (58.0 ± 10.6 vs 27.8 ± 4.3, $P < .02$). Fasting plasma C-reactive protein (7787 ± 884 vs 236 ± 79 ng/mL, $P < .0001$) and TNF-α (2.37 ± 0.09 vs 0.54 ± 0.04 pg/mL, $P < .05$) were both elevated in obese women. Hyperglycemia resulted in a suppression of LPS-stimulated TNF-α release from MNCs of normal-weight subjects (154 ± 21 vs 57 ± 28 pg/mL, $P < .003$), but no change in obese women (148 ± 36 vs 173 ± 49 pg/mL). The TNF-α response was different between groups (−97 ± 21 vs +24 ± 22 pg/mL, $P < .003$). There was also a positive association between the incremental change in MNC-derived TNF-α and percentage of truncal fat ($r = 0.75$, $P < .002$). In conclusion, these data suggest that there is an absence of the "normal" suppression of TNF-α in MNCs after hyperglycemia in obese women, and this response may contribute to impaired glucose disposal and insulin resistance.
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1. Introduction

Obesity is associated with the development of insulin resistance and hyperglycemia [1,2]. Obesity is also a pro-inflammatory state as evidenced by elevated plasma concentrations of C-reactive protein (CRP) [3,4]. Some studies have also reported plasma elevations in the pro-inflammatory cytokine, tumor necrosis factor α (TNF-α), in obese subjects [3,5,6]. This has not been confirmed by other investigations and thus remains controversial [7-10]. However, there is sufficient evidence of TNF-α expression in multiple tissues where TNF-α exerts its effect in an autocrine-paracrine fashion typical of cytokines [11,12]. Several human and animal studies have documented overexpression of TNF-α in adipose tissue when obesity or type 2 diabetes mellitus is present [13-15]. Tumor necrosis factor α may induce insulin resistance through short- and long-term effects on insulin-sensitive tissues. Moreover, in vitro studies have demonstrated that TNF-α immediately truncates insulin receptor signaling in cultured adipocytes, hepatocytes, and skeletal muscle [16-18]. In addition, long-term exposure to TNF-α impairs mobilization of GLUT 4, the insulin-sensitive glucose transport protein [19].

It has recently been shown that peripheral blood mononuclear cells (MNCs) of obese subjects are activated in a pro-inflammatory state [20]. This is important because MNCs are known to migrate into adipose tissue to activate adipocyte TNF-α production [21,22]. However, it is now

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clear that the major source of TNF-α in adipose tissue of obese subjects is MNC-derived macrophages present in the stromal-vascular compartment [22-25]. Mononuclear cells exhibit increased oxidative stress in response to hyperglycemia, which is known to activate nuclear factor κB (NFκB), a pro-inflammatory transcription factor that promotes TNF-α gene transcription [26-28]. We have previously reported that in response to hyperglycemia, the increased abdominal adiposity of insulin-resistant older men was related to increased lipopolysaccharide (LPS)-stimulated TNF-α release from MNCs [29]. However, this relationship has never been explored in obese reproductive-age women.

Thus, we embarked on a study to determine the status of TNF-α release from MNCs in response to hyperglycemia in obese reproductive-age women. It is important to characterize this phenomenon in women who are at greater risk of developing metabolic abnormalities affecting reproduction. Moreover, obese reproductive-age women are more likely to develop gestational diabetes and other obesity-related obstetrical complications [30-32]. They may also exhibit phenotypic expression of polycystic ovary syndrome in individuals who are susceptible [33,34]. We hypothesized that LPS-stimulated TNF-α release from MNCs is altered in obese reproductive-age women in response to an oral glucose challenge as compared with normal-weight women of similar age, and that there is a relationship between MNC-derived TNF-α release and measures of adiposity.

2. Materials and methods

2.1. Subjects

Fourteen women (6 obese and 8 normal weight) aged between 20 and 40 years participated in the study. Obesity was defined as a body mass index (BMI) between 30 and 40 kg/m^2. Normal-weight subjects had a BMI between 18 and 25 kg/m^2. All subjects were ovulatory as evidenced by regular menses and a luteal-phase serum progesterone level greater than 5 ng/mL. All subjects were screened for diabetes, inflammatory illnesses, or endocrinopathies, and none were taking medications that would affect carbohydrate metabolism or immune function. Based on Adult Treatment Panel III guidelines to diagnose the metabolic syndrome, none of the subjects exhibited 3 or more of the following features: waist circumference of more than 88 cm, plasma triglyceride of 150 mg/dL or higher, plasma high-density lipoprotein cholesterol (HDL-C) of less than 50 mg/dL, blood pressure $\geq 130/\geq 85$ mm Hg, and fasting glucose of 110 mg/dL or higher [35]. 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 MetroHealth Medical Center guidelines for the protection of human subjects.

2.2. Study design

All study subjects underwent an oral glucose tolerance test (OGTT) between 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). The test was performed on the morning of day 4 after an overnight fast of ~12 hours. All subjects also underwent body composition assessment on the same day the OGTT was performed.

2.3. Oral glucose tolerance test

Baseline blood samples (5 mL each) were drawn for glucose and insulin determination. A 75-g glucose beverage was subsequently ingested over 10 minutes. Blood samples (5 mL each) were again drawn for glucose and insulin determination 2 hours 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 same blood samples and stored at −70°C until assayed for CRP, TNF-α, and lipids. Glucose tolerance was assessed by the World Health Organization criteria with normal glucose tolerance defined as a 2-hour glucose stimulated value less than 140 mg/dL [36]. Insulin sensitivity was estimated by homeostasis model assessment of insulin resistance (IS_{HOMA}) using the following formula: fasting glucose × fasting insulin/22.5 [37].

2.4. 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 [38]. In addition, all subjects underwent dual-energy absorptiometry to determine the percentage of total body fat and percentage of truncal fat using the QDR 4500 Elite model scanner (Hologic, Waltham, MA). 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) [39].

2.5. Analytical methods

Mononuclear cell isolation and culture were performed on a 20-mL blood sample drawn at 0 (pre) and 2 (post) hours during the OGTT. The cells were isolated by Histopaque-1077 density gradient centrifugation [40], washed 2 times in pyrogen-free saline, resuspended in RPMI (0.3 mg/mL L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin) with serum substitute TCH (TCH Serum Replacement, MD Biomedicals Inc, Irvine, CA), and seeded in coated culture plates (2.5×10^6 cells/mL). The cells were then incubated (humidified 5% CO_2, 37°C) for 24 hours with LPS endotoxin (1 ng/mL). Cell supernatants (10 000g for 2 minutes) were subsequently collected and stored at −70°C until analysis.

Table 1
Body composition and fasting lipid levels of subjects

	Normal weight (n = 8)	Obese (n = 6)
Age (y)	33 ± 2	30 ± 3
Systolic blood pressure (mm Hg)	104 ± 3	113 ± 5
Diastolic blood pressure (mm Hg)	58 ± 3	73 ± 6*
Height (cm)	165 ± 1.1	162 ± 3.0
Body weight (kg)	59.7 ± 1.9	89.2 ± 3.2*
BMI (kg/m^2)	21.9 ± 0.8	34.1 ± 1.1*
Total body fat (%)	28.7 ± 1.8	42.4 ± 1.3*
Truncal fat (%)	25.4 ± 2.5	42.1 ± 1.2*
Waist circumference (cm)	74.3 ± 2.9	101.6 ± 3.5*
Total cholesterol (mg/dL)	171 ± 7	214 ± 23
Triglyceride (mg/dL)	54 ± 6	132 ± 50
HDL-C (mg/dL)	53 ± 4	52 ± 4
LDL cholesterol (mg/dL)	111 ± 6	145 ± 15*

Values are expressed as means ± SE.
* $P < .002$, significantly different from the lean group.

Plasma glucose concentrations were measured by the glucose oxidase method (YSI, Yellow Springs, OH), whereas plasma insulin concentrations were measured by a double-antibody radioimmunoassay (Linco Research, St Charles, MO). Plasma CRP concentrations were measured by a high-sensitivity enzyme-linked immunosorbent assay (Alpha Diagnostics International, San Antonio, TX). Tumor necrosis factor α concentrations were also measured by enzyme-linked immunosorbent assay (BioSource International, Camarillo, CA). Levels of total cholesterol, triglyceride, and HDL-C were measured by enzymatic methods (SYNCHRON LX20 PRO automatic analyzer, Beckman Coulter, Fullerton, CA). Low-density lipoprotein (LDL) cholesterol was calculated using the formula of Friedewald et al [41]. All samples from each subject were measured in duplicate in the same assay at the end of the study. The interassay and intra-assay coefficients of variation for all assays were 7% and 12%, respectively.

Table 2
Plasma glucose, insulin, CRP, and TNF-α levels, and TNF-α release from MNCs while fasting and in response to an oral glucose challenge in lean and obese women

	Fasting	2 h postglucose	Δ
Glucose (mg/dL)			
Normal weight	85.9 ± 1.6	98.5 ± 8.9	12.6 ± 8.4
Obese	83.8 ± 5.1	121.3 ± 6.1*	36.5 ± 10.9
Insulin (μU/mL)			
Normal weight	7.3 ± 1.2	32.2 ± 6.5*	24.9 ± 5.7
Obese	15.0 ± 2.4†	98.5 ± 25.9*	83.5 ± 24.7
CRP (ng/mL)			
Normal weight	236 ± 79	239 ± 87	3 ± 24
Obese	7787 ± 884†	7209 ± 1020†	578 ± 200
Plasma TNF-α (pg/mL)			
Normal weight	0.55 ± 0.04	0.57 ± 0.04	0.02 ± 0.05
Obese	2.37 ± 0.93†	2.13 ± 1.10	−0.24 ± 0.28
MNC TNF-α (pg/mL)			
Normal weight	154 ± 21	57 ± 28#	−97 ± 21
Obese	148 ± 36	173 ± 49	25 ± 22‡

Values are expressed as means ± SE. Δ indicates calculated difference between means for 2 hours postglucose-fasting.
* $P < .02$, 2 hours postglucose significantly higher than fasting.
† $P < .05$, significantly higher than the lean group.
# $P < .003$, 2 hours postglucose significantly lower than fasting.
‡ $P < .003$, significantly different response than the lean group.

2.6. Statistics

The StatView statistical package (SAS Institute, Cary, NC) was used for data analysis. The difference between the pre- and postglucose challenge values for primary dependent variables such as TNF-α release from MNCs was calculated to represent the incremental change. Descriptive data and the incremental change of variables were compared between groups using the unpaired Student *t* test. Differences between pre- and postglucose challenge variables within groups were analyzed using the paired Student *t* test. Correlation analyses were performed by linear regression using the method of least squares. All values are expressed as means ± SE. An α level of .05 was used to determine statistical significance.

3. Results

Age and height were similar between groups, and all subjects were normotensive. The obese group had significantly higher ($P < .002$) weight, BMI, percentage of total body fat, percentage of truncal fat, waist circumference, and LDL levels (Table 1).

Levels of glucose while fasting and 2 hours postglucose ingestion were similar in both groups (Table 2). All subjects had a normal glucose response during the OGTT with 2-hour glucose levels between 62 and 138 mg/dL. However, fasting insulin levels were significantly higher ($P < .05$) in the obese group. In addition, the 2-hour glucose level in the obese group and the 2-hour insulin level in both groups were significantly increased ($P < .02$) compared with fasting levels. As depicted in Fig. 1, IS_{HOMA} was significantly greater ($P < .02$) in the obese group compared with the lean control group. IS_{HOMA} was positively correlated with BMI ($r = 0.65$, $P < .02$), percentage of total body fat ($r = 0.53$, $P < .05$), percentage of truncal fat ($r = 0.54$, $P < .05$), and waist circumference ($r = 0.69$, $P < .009$) for the combined groups (data not shown).

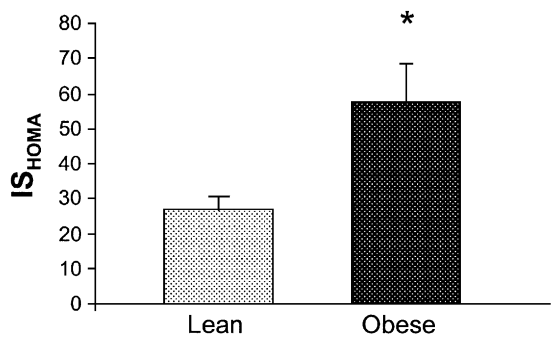

Fig. 1. Estimate of insulin resistance based on IS_{HOMA}. *$P < .02$, significantly greater insulin resistance in the obese group.

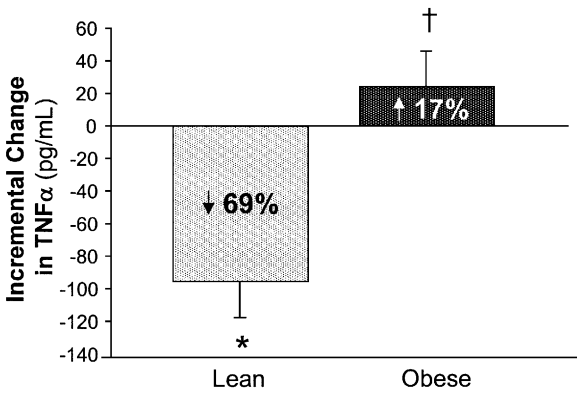

Fig. 2. Incremental change in TNF-α release (pg/mL) from MNCs cultured with LPS for 24 hours when fasting samples (pre) were compared with the samples collected 2 hours after glucose ingestion (post). *$P < .003$, 2 hours postglucose was significantly lower than fasting in the normal-weight group. †$P < .003$, TNF-α response to oral glucose challenge in the obese group was significantly different from that of the normal-weight group.

Fasting plasma concentrations of CRP and TNF-α were significantly ($P < .05$) higher in the obese group, but remained unchanged after glucose ingestion in both groups (Table 2). Lipopolysaccharide-stimulated TNF-α release from MNCs in the fasting state was also similar in both groups. However, hyperglycemia resulted in significant ($P < .003$) suppression of LPS-stimulated TNF-α release from MNCs of normal-weight controls, but no change in obese women (Table 2 and Fig. 2). In addition, the incremental change in TNF-α release from MNCs between the 2 groups was significantly different ($P < .003$). As depicted in Fig. 3, there was a direct relationship between the MNC-derived incremental change in TNF-α release and percentage of truncal fat ($r = 0.75$, $P < .002$) for the combined groups. There was also a direct relationship between the MNC-derived incremental change in TNF-α release and BMI ($r = 0.66$, $P < .01$), percentage of total body fat ($r = 0.74$, $P < .003$), and waist circumference ($r = 0.78$, $P < .002$) for the combined groups (data not shown).

4. Discussion

Our data clearly show that obese reproductive-age women have impaired suppression of TNF-α release from MNCs in response to physiological hyperglycemia. In contrast, TNF-α release from MNCs was suppressed in normal-weight controls of similar age under postprandial-like conditions. Because TNF-α is a pro-inflammatory cytokine, these findings provide further support for the role of inflammation in the development of insulin resistance, and that TNF-α particularly may contribute to the decline in insulin action in obese reproductive-age women. Furthermore, the independent associations of TNF-α with BMI, percentage of total body fat, percentage of truncal fat, and waist circumference suggest that increased adiposity, especially increased abdominal adiposity, may be a key determinant of the observed differences in the MNC-derived TNF-α response and its potential role in modulating insulin action.

The normal in vivo response of MNCs to physiological hyperglycemia may be to suppress the release of TNF-α. Lean controls in the present study showed a 69% decrease in LPS-stimulated TNF-α release from MNCs in response to hyperglycemia. We have previously reported similar results in healthy normal-weight young men [29]. Decreased TNF-α release from MNCs may be a physiological benefit in the presence of hyperglycemia when there is a need to increase glucose disposal. Tumor necrosis factor α is known to cause a decrease in insulin receptor tyrosine phosphorylation and an increase in serine phosphorylation of insulin receptor substrate 1, leading to inhibition of downstream insulin signaling and impairment of glucose uptake [13,16]. Thus, normal-weight individuals may be capable of facilitating glucose disposal by controlling TNF-α release to optimize insulin signaling in the postprandial state.

In contrast, the MNCs of obese reproductive-age women may have an impaired ability to down-regulate TNF-α release in response to physiological hyperglycemia. Indeed, the elevated plasma concentrations of CRP and TNF-α observed in this group confirm previous reports demonstrating that obesity is a pro-inflammatory state [3-6]. Obese reproductive-age women also exhibited evidence of insulin resistance based on the increase in IS_{HOMA}. In vivo oral glucose challenge has been shown to stimulate a transient increase in reactive oxygen species generation from MNCs of normal individuals resulting in oxidative stress [27]. Similar pro-inflammatory responses have been noted after lipid and protein intakes [42,43]. Oxidative stress causes activation and nuclear translocation of NFκB to promote transcription of a variety of inflammatory

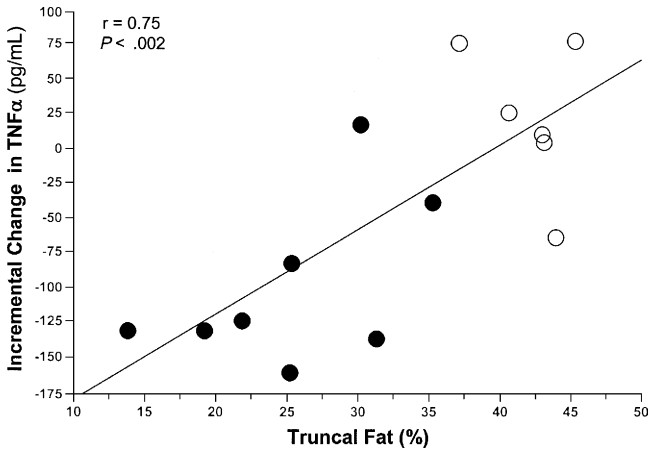

Fig. 3. Correlation between abdominal adiposity and the incremental change in TNF-α release from MNCs after oral glucose challenge. Data are shown for 14 women of reproductive age with normal glucose tolerance. Tumor necrosis factor α release was measured from LPS-stimulated MNCs obtained at 0 and 2 hours of an oral glucose challenge test. Abdominal obesity was estimated from the percentage of truncal fat measured by dual-energy absorptiometry. Open circles indicate obese group; closed circles, normal-weight control group.

mediators including TNF-α [26,28]. It is possible that continuously increased macronutrient intake leading to obesity results in an increase in oxidative stress and inflammation observed in the obese. A subsequent failure to suppress TNF-α release from MNCs in the postprandial state may promote insulin resistance. This concept is further supported by previous reports of a reduction in oxidative stress and inflammatory mediators after energy restriction in obese subjects and after a 2-day fast in healthy subjects [5,44,45].

Our data suggest that there may be a link between obesity and MNC-derived TNF-α release. There was a direct relationship between the change in TNF-α release from MNCs after physiological hyperglycemia and measures of adiposity, particularly abdominal adiposity. Obese reproductive-age women with insulin resistance exhibited increased BMI, percentage of total body fat, percentage of truncal fat, and waist circumference. Activated MNC-derived macrophages are the major source of TNF-α in the increased adipose tissue of the obese and are capable of inducing further TNF-α production in adipocytes [22-25]. It is possible that the inflamed adipose tissue of the obese, especially in the abdominal region, perpetuates the inability to suppress MNC-derived TNF-α release after hyperglycemia. These findings are consistent with previous observations in young adults demonstrating that changes in insulin sensitivity are a function of abdominal adiposity [31,46]. Thus, the uncontrolled TNF-α release may, in turn, promote the insulin resistance observed in our obese subjects.

The hyperinsulinemia that occurs after an oral glucose challenge is unlikely to contribute to the unsuppressed TNF-α release from MNCs in obese subjects. In a previous study, infusion of insulin as opposed to that of saline serving as a control suppressed reactive oxygen species generation and NFκB activation in the obese [47]. Thus, insulin exerts an anti-inflammatory effect that ameliorates the pro-inflammatory response to physiological hyperglycemia evident in an insulin-resistant state such as obesity.

In conclusion, obese reproductive-age women exhibited an altered MNC-derived TNF-α response during physiological hyperglycemia. The failure of these obese subjects to suppress LPS-stimulated TNF-α release compared with a normal-weight control group suggests that TNF-α of MNC origin is involved in mediating insulin resistance observed in these individuals. Our findings also demonstrate that the increased adiposity in obese young women promotes a pro-inflammatory state. The association between the change in TNF-α release and both total and abdominal fat suggests that increased adiposity contributes to the different TNF-α responses in these groups. We recognize that our findings obtained from LPS stimulation may not reflect the physiological circumstance. However, it is intriguing to consider the possibility that when glucose increases during the postprandial period in normal-weight individuals, TNF-α release from MNCs is decreased to facilitate glucose uptake by insulin-sensitive tissues. Conversely, the loss of this postprandial response may be one of the factors that contribute to insulin resistance in the obese subjects.

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