Metabolic syndrome and endothelial fibrinolytic capacity in obese adults

Gary P. Van Guilder,1 Greta L. Hoetzer,1 Jared J. Greiner,1 Brian L. Stauffer,1,2,3 and Christopher A. DeSouza1,2

1Integrative Vascular Biology Laboratory, Department of Integrative Physiology, University of Colorado, Boulder; and 2Department of Medicine, University of Colorado, Health Sciences Center, Denver; and 3Denver Health Medical Center, Denver, Colorado

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Van Guilder GP, Hoetzer GL, Greiner JJ, Stauffer BL, DeSouza CA. Metabolic syndrome and endothelial fibrinolytic capacity in obese adults. Am J Physiol Regul Integr Comp Physiol 294: R39–R44, 2008. First published October 24, 2007; doi:10.1152/ajpregu.00564.2007.— The metabolic syndrome (MetS) often accompanies obesity and contributes to the increased risk of atherothrombotic events with increased body fatness. Indeed, the risks for coronary artery disease and acute vascular events are greater with obesity combined with MetS compared with obesity alone. Endothelial release of tissue-type plasminogen activator (t-PA) is a key defense mechanism against thrombosis and has been shown to be impaired with obesity. The aim of the present study was to determine whether the presence of MetS exacerbates endothelial fibrinolytic dysfunction in obese adults. Net endothelial release of t-PA was determined in vivo in response to intrabrachial infusions of bradykinin and sodium nitroprusside in 47 sedentary adults: 15 normal weight (age 57 ± 2 yr; body mass index 22.9 ± 0.5 kg/m²), 14 obese but otherwise healthy (55 ± 1 yr; 29.4 ± 0.3 kg/m²), and 18 obese with MetS (55 ± 2 yr; 32.3 ± 1 kg/m²). MetS was established according to National Cholesterol Education Program ATP III criteria. Net release of t-PA antigen to bradykinin and fibrinolytic factors resulting in a prothrombotic state are now considered to be a component of the MetS (23, 26, 38). With respect to the fibrinolytic system, plasma concentrations of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) have been shown to be elevated with the MetS, suggesting reduced endogenous fibrinolytic activity (2, 3, 40, 44). However, plasma concentrations of fibrinolytic proteins provide an indirect, nonspecific, and potentially misleading assessment of fibrinolytic potential. It is the capacity of the vascular endothelium to acutely and rapidly release t-PA and not circulating plasma fibrinolytic concentrations that determines the effectiveness of endogenous fibrinolysis (20, 37, 45). We have previously demonstrated that the capacity of the endothelium to release t-PA is markedly blunted in overweight and obese adults free of cardiovascular and metabolic disorders (46). Although the influence of the MetS on plasma markers of fibrinolysis in obese adults has been well studied (2, 21, 22, 26, 44), there is no information regarding the impact of the MetS on endothelial t-PA release in this population.

Accordingly, the aim of the present study was to determine whether the presence of the MetS exacerbates endothelial fibrinolytic dysfunction in obese adults. We hypothesized that obesity combined with the MetS would be associated with a greater impairment in endothelial t-PA release compared with obesity independent of the MetS. To address this aim, we used an isolated forearm model to assess in vivo rates of endothelial t-PA release in obese adults with and without the MetS.

METHODS

Subjects

Forty-seven sedentary adults (33 males, 14 females) ranging in age from 45 to 71 yr were studied: 15 normal-weight and 32 obese subjects [14 without MetS and 18 with MetS (obese/MetS)]. In the present study, subjects with a body mass index (BMI) ≥ 25 kg/m² were classified as obese. We have previously demonstrated no difference in endothelial t-PA release between overweight (BMI ≥ 25 < 30 kg/m²) and obese adults (BMI ≥ 30 kg/m²). MetS was established according to the National Cholesterol Education Program ATP III criteria (14, 15, 32a). Our rationale for selecting the ATP III criteria was based on data from the San Antonio Study, suggesting that the ATP III criteria was superior to the World Health Organization definition (47) for predicting cardiovascular disease and diabetes (17, 28). All subjects were sedentary and had not participated in a regular aerobic exercise program for ≥1 yr before the start of the study. Subjects were nonsmokers, nonmedicated (including vitamins), nondiabetic, and free of overt cardiovascular disease as assessed by medical history.

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physical examination, resting, exercise electrocardiograms, and fasting blood chemistries. All of the women in the study were at least \( \geq 1 \) yr postmenopausal (mean: 9 \( \pm \) 2 yr) and had never taken or discontinued hormone replacement therapy \( \geq 1 \) yr before the start of the study. Before participation, all of the subjects had the research study and its potential risks and benefits explained fully before providing written informed consent. This study was approved by the Human Research Committee of the University of Colorado at Boulder.

**Measurements**

**Body composition.** Body mass was measured to the nearest 0.1 kg using a medical beam balance (Detecto, Webb City, MO). Percent body fat was determined by dual-energy X-ray absorptiometry (Lunar Radiation, Madison, WI). BMI was calculated as weight (kg) divided by height (m) squared. Minimal waist circumference was measured according to previously published guidelines (27).

**Treadmill exercise test.** To assess aerobic fitness, subjects performed incremental treadmill exercise using a modified Balke protocol as previously described (10). Maximal oxygen consumption \( (\dot{V}O_2_{\text{max}}) \) was measured using online, computer-assisted, open circuit spirometry.

**Metabolic measurements.** Fasting plasma lipid and lipoprotein, glucose, and insulin concentrations were determined using standard techniques by the clinical laboratory affiliated with the General Clinical Research Center. Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR) derived from fasting glucose and insulin concentrations (31).

**Intra-Arterial Fibrinolytic Protocol**

All measurements were performed between 7 and 10 AM after a 12-h overnight fast, as previously described by our laboratory (42). Briefly, an intravenous catheter was placed in an antecubital vein of the nondominant arm. Thereafter, a 5-cm, 20-gauge catheter was introduced into the brachial artery of the same arm under local anesthesia (1% lidocaine). Forearm blood flow (FBF) was measured using strain-gauge venous occlusion plethysmography (D. E. Hokanson, Bellevue, WA) and presented as milliliters per 100 milliliters forearm volume per minute. Following the measurement of resting blood flow for 5 min, bradykinin was infused intra-arterially at 12.5, 25, and 50 ng \( \cdot \) 100 ml tissue \( \cdot \) min \(^{-1} \) and sodium nitroprusside at 1.0, 2.0 and 4.0 \( \mu \)g \( \cdot \) 100 ml tissue \( \cdot \) min \(^{-1} \) for 5 min at each dose. To avoid an order effect, the sequence of drug administration was randomized. Forearm volume was determined by the water displacement method.

Net endothelial release of t-PA and PAI-1 antigen in response to bradykinin and sodium nitroprusside was calculated using the following equation (5, 20). Net Release \( = \) \( (C_0 - C_E) \times [\text{FBF} \times (101 - \text{hematocrit/100})] \), where \( C_0 \) and \( C_E \) represent the concentration in the vein and artery, respectively. For both t-PA and PAI-1, a positive difference indicated a net release and a negative difference (net uptake). Arterial and venous blood samples were collected simultaneously at baseline and at the end of each drug dose, t-PA and PAI-1 antigen concentrations were determined by enzyme immunoassay. Hematocrit was measured in triplicate using the standard microhematocrit technique and corrected for trapped plasma volume within the trapped erythrocytes (7). The total amount of t-PA antigen released across the forearm in response to all three doses of bradykinin was calculated as the total area under each curve above baseline using a trapezoidal model. To avoid confounding effects from potential infection/inflammation-associated fibrinolytic changes, all subjects were free of recent infection/inflammation (<2 wk), as determined by questionnaire (29).

**Statistical Analysis**

Differences in subject baseline characteristics and area under the curve data were determined by between-groups ANOVA. Group differences in FBF and endothelial t-PA and PAI-1 antigen release in response to bradykinin and sodium nitroprusside were determined by repeated-measures ANOVA. When indicated by a significant \( F \) value, a post hoc test using the Newman-Keuls method was performed to identify differences between the groups. Relations between variables of interest were assessed by means of Pearson’s correlation coefficient and linear regression analysis. There were no significant gender interactions in any of the primary outcome variables; therefore, the data were pooled and presented together. All data are expressed as means \( \pm \) SE. Statistical significance was set a priori at \( P < 0.05 \).

**RESULTS**

Table 1 presents selected subject characteristics. There were no differences in age, diastolic blood pressure, \( \dot{V}O_2_{\text{max}} \), and total cholesterol between the groups. As expected, body mass, BMI, percent body fat, and waist circumference were higher \( (P < 0.01) \) in obese adults with and without MetS compared with normal-weight controls. In addition to indices of adiposity, high-density lipoprotein cholesterol was lowest; and systolic blood pressure, plasma triglycerides, glucose, and insulin, as well as HOMA-IR were highest \( (all \ P < 0.01) \) in obese/MetS adults. Fasting plasma concentrations of t-PA and PAI-1 antigen were also higher in the obese/MetS adults. There were no significant differences in either t-PA antigen or PAI-1 antigen concentrations between the normal-weight and obese groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Weight</th>
<th>Obese</th>
<th>Obese/MetS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>15</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Age, yr</td>
<td>57 ( \pm ) 2</td>
<td>55 ( \pm ) 1</td>
<td>55 ( \pm ) 2</td>
</tr>
<tr>
<td>Sex, M/W</td>
<td>11/4</td>
<td>9/5</td>
<td>13/5</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>69.9 ( \pm ) 1.8</td>
<td>85.3 ( \pm ) 1.9*</td>
<td>99.0 ( \pm ) 3.5†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.9 ( \pm ) 0.5</td>
<td>29.4 ( \pm ) 0.3*</td>
<td>32.3 ( \pm ) 0.9†</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>23.6 ( \pm ) 2.4</td>
<td>34.4 ( \pm ) 2.2*</td>
<td>37.1 ( \pm ) 1.7†</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>82.9 ( \pm ) 2.5</td>
<td>98.3 ( \pm ) 1.7*</td>
<td>107.7 ( \pm ) 2.6†</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>119 ( \pm ) 3</td>
<td>119 ( \pm ) 3</td>
<td>128 ( \pm ) 2†</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>77 ( \pm ) 2</td>
<td>78 ( \pm ) 2</td>
<td>82 ( \pm ) 2</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.7 ( \pm ) 0.2</td>
<td>4.7 ( \pm ) 0.3</td>
<td>5.2 ( \pm ) 0.3</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/l</td>
<td>2.9 ( \pm ) 0.2</td>
<td>2.9 ( \pm ) 0.2</td>
<td>3.2 ( \pm ) 0.2</td>
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<tr>
<td>HDL-cholesterol, mmol/l</td>
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<td>1.3 ( \pm ) 0.1</td>
<td>1.0 ( \pm ) 0.1†</td>
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<tr>
<td>Triglycerides, mmol/l</td>
<td>1.0 ( \pm ) 0.1</td>
<td>1.2 ( \pm ) 0.1</td>
<td>2.1 ( \pm ) 0.1†</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.9 ( \pm ) 0.1</td>
<td>5.0 ( \pm ) 0.1</td>
<td>5.5 ( \pm ) 0.1†</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>27.6 ( \pm ) 3.1</td>
<td>34.8 ( \pm ) 3.3*</td>
<td>56.4 ( \pm ) 6.9*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.8 ( \pm ) 0.7</td>
<td>1.3 ( \pm ) 0.2*</td>
<td>2.3 ( \pm ) 0.3†</td>
</tr>
<tr>
<td>t-PA antigen, ng/ml</td>
<td>9.5 ( \pm ) 1.2</td>
<td>10.5 ( \pm ) 0.9</td>
<td>11.6 ( \pm ) 0.9*</td>
</tr>
<tr>
<td>PAI-1 antigen, ng/ml</td>
<td>9.0 ( \pm ) 1.8</td>
<td>11.9 ( \pm ) 2.1</td>
<td>26.4 ( \pm ) 5.7†</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. M, men; W, women; BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostasis model of insulin resistance; t-PA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1. *\( P < 0.05 \) vs. normal weight; †\( P < 0.05 \) vs. obese.
MetS subjects there were no significant differences among the groups (Fig. 1). Basal endothelial t-PA antigen release was not significantly different among the groups. However, the capacity of the endothelium to release t-PA in response to bradykinin was significantly blunted in the obese subjects with and without the MetS (Fig. 2). Net release of t-PA antigen was ~50% lower ($P < 0.01$) in the obese (from 2.5 ± 1.9 to 37.1 ± 5.3 ng·100 ml tissue$^{-1}$·min$^{-1}$) and obese/MetS (from 0.4 ± 0.8 to 32.5 ± 3.8 ng·100 ml tissue$^{-1}$·min$^{-1}$) compared with normal-weight (from 0.9 ± 1.0 to 74.3 ± 8.1 ng·100 ml tissue$^{-1}$·min$^{-1}$) subjects. As a result, the total amount of t-PA antigen released (area under the curve to all doses of bradykinin) was markedly lower (~40%; $P < 0.01$) in the obese (164 ± 26 ng/100 ml tissue) and obese/MetS (187 ± 19 ng/100 ml tissue) compared with normal-weight (360 ± 41 ng/100 ml tissue) adults (Fig. 1). Of note, there were no significant differences in either the rate or total amount of t-PA released between the obese without MetS and obese/MetS adults. Infusion of sodium nitroprusside did not stimulate significant changes in t-PA release in either the normal-weight (from −0.7 ± 0.7 to 7.4 ± 7.3 ng·100 ml tissue$^{-1}$·min$^{-1}$), obese (from 0.7 ± 2.3 to 5.2 ± 6.3 ng·100 ml tissue$^{-1}$·min$^{-1}$), or obese/MetS (from 0.4 ± 0.9 to 1.8 ± 3.0 ng·100 ml tissue$^{-1}$·min$^{-1}$) groups. Table 2 shows the venous-arterial concentration differences for t-PA antigen in response to both bradykinin and sodium nitroprusside. Neither bradykinin nor sodium nitroprusside elicited significant changes in PAI-1 antigen release in either group (data not shown).

Fig. 1. Forearm blood flow responses to bradykinin and sodium nitroprusside in normal-weight, obese, and obese/MetS adults. MetS, metabolic syndrome. Values are means ± SE; $P < 0.05$ obese groups vs. normal weight.

Fig. 2. Net release rate and total amount of tissue-type plasminogen activator (t-PA) antigen released (area under the curve) across the forearm in response to bradykinin in normal-weight, obese and obese/MetS adults. Values are means ± SE; *$P < 0.05$ vs. normal weight.
In the overall study population, the total amount of t-PA antigen released in response to bradykinin was significantly related to body mass (r = -0.42), BMI (r = -0.52), percent fat (r = -0.35), waist circumference (r = -0.38), plasma triglycerides (r = -0.35), plasma glucose (r = -0.40), plasma insulin (r = -0.41), and HOMA-IR (r = -0.41). In the obese and obese/MetS groups percent fat was the only significant correlate of t-PA release (r = -0.68 and r = -0.62, respectively).

DISCUSSION
The primary new finding of the present study is that the presence of the MetS does not worsen endothelial fibrinolytic dysfunction in obese adults. Contrary to our hypothesis, we observed similar rates and total amounts of t-PA released in response to bradykinin in obese adults with and without the MetS. To our knowledge, this is the first study to determine the influence of the MetS on endothelial t-PA release in obese adults.

The capacity of the endothelium to release t-PA is an important endogenous defense mechanism against intravascular fibrin deposition and thrombosis (32). The thrombolytic potential of t-PA is greatest if it is locally released in large quantities to be incorporated during, rather than after, thrombus formation (4, 12). Given that the inhibitory interaction between PAI-1 and t-PA has a second-order rate constant of \( \sim 10^9 \text{M}^{-1} \text{s}^{-1} \) (36), local rapid release of t-PA from the endothelium is absolutely critical to the fibrinolytic process. Animal studies have shown that the ability of the endothelium to locally and rapidly release t-PA in response to a thrombotic stimulus results in fibrin degradation and clot resolution (13, 24), whereas, deficiencies in t-PA release are associated with accelerated atherosclerosis with uncontrolled luminal fibrin deposition and severe myocardial tissue necrosis (6, 8). In humans, reduced coronary endothelial t-PA release has been linked to increased atheromatous plaque burden (33, 34) and myocardial infarction (16). The development of a hypofibrinolytic, prothrombotic state is now considered to be a feature of the MetS, contributing to the increased risk of acute cardiovascular events with this pathology (2, 3, 44). The salient and novel finding of the present study is that the ability of the endothelium to acutely release t-PA is unaffected by the presence of the MetS in obese adults. The magnitude of the increase in net endothelial release of t-PA in response to bradykinin in the obese/MetS adults, while significantly lower (~45%) than the normal-weight subjects, was similar to the obese adults without the MetS. The fact that we observed comparable levels of endothelial t-PA release despite higher basal plasma concentrations of t-PA and PAI-1 antigen in obese adults with MetS compared with those without MetS provides further evidence that circulating fibrinolytic factors do not reflect the ability of the endothelium to release t-PA and, in turn, endogenous fibrinolytic potential (20, 42). Indeed, although circulating PAI-1 antigen concentrations were higher in the obese and obese/MetS groups, baseline t-PA release rates were not different among the groups ruling out the potential confounding effects of t-PA/PAI-1 complex formation on our results. Moreover, we observed no differences in the net release of PAI-1 antigen (to either bradykinin or sodium nitroprusside) between the groups, thus ruling out the possibility that elevated PAI-1 antigen levels blunted t-PA release in the obese groups. Collectively, our findings do not support the notion that impaired fibrinolytic capacity is an independent component of the MetS (3, 48, 49) but rather that fibrinolytic dysfunction is a consequence of obesity not the MetS.

Obesity and insulin resistance, which often occur together, are considered to be major underlying risk factors in the etiology of the MetS (9, 15). The results of the present study suggest that the capacity of the endothelium to release t-PA is affected more by adiposity than the presence of either insulin resistance or the MetS. Indeed, although the obese adults with MetS were more insulin resistant and demonstrated a less favorable cardiometabolic risk profile than the obese subjects without the MetS, endothelial t-PA release was not different among the groups. Moreover, in both obese groups percent body fat was the only significant correlate of t-PA release. It should be noted that the obese/MetS subjects in the present study demonstrated hemodynamic and metabolic characteristics that were not grossly abnormal; moreover, none of our subjects were hypertensive or diabetic. Thus, we cannot dismiss the possibility that a more severe atherogenic cardiometabolic risk profile may have resulted in greater impairment in endothelial t-PA release in the obese/MetS adults.

In the present study, we defined the presence of the MetS according to National Cholesterol Education Program (NCEP) ATP III guidelines (14, 32a). To determine whether our results were specific to this MetS criteria, we reclassified our study population according to the International Diabetes Federation definition of MetS (1). Using International Diabetes Federation criteria for defining MetS, we observed remarkably similar results to those reported herein. There was no difference in endothelial t-PA release rates to bradykinin between obese subjects with (from 1.9 ± 1.8 to 34.1 ± 5.1 ng·100 ml tissue⁻¹·min⁻¹; n = 16) and without (from 0.7 ± 0.7 to 34.8 ± 5.8 ng·100 ml tissue⁻¹·min⁻¹; n = 16) the MetS; whereas both groups demonstrated markedly blunted t-PA release compared with normal-weight controls (from 0.9 ± 1.0 to 74.3 ± 8.1 ng·100 ml tissue⁻¹·min⁻¹; n = 15). We did not
reanalyze our data using the World Health Organization definition of MetS due to its requirement for a measure of insulin resistance, preferably by euglycemic clamp (47). Nevertheless, the fact that we observed comparable results using two established definitions of MetS buttresses the postulate that endothelial fibrinolytic capacity in obese adults is not adversely affected by the presence of the MetS.

There are a number of important experimental considerations regarding this study that should be mentioned. First, as with all cross-sectional study designs, it is possible that genetic and/or lifestyle behaviors may have influenced the results of our group comparisons. In an effort to minimize the influence of lifestyle behaviors, we studied normal-weight and obese adults (with and without MetS) of similar age who were nonsmokers, not currently taking medication that could influence endothelial fibrinolytic activity, and did not differ in habitual physical activity. In addition, we employed strict inclusion criteria to eliminate the confounding effects of clinically overt cardiovascular and metabolic disease. Second, all of the subjects in the present study were Caucasian, thus our results cannot be generalized to other ethnic groups (39). Because of the substantial racial and ethnic variability in susceptibility to, and prevalence of, risk factors associated with the MetS (41), our findings should be viewed in the context of the population studied. Third, because we did not study normal-weight adults with the MetS we cannot discount the possibility that MetS, independent of obesity, may have adverse effects on endothelial fibrinolytic regulation. However, since the vast majority of individuals with the MetS are overweight or obese, we believe that our results pertain to a broader at-risk clinical population. Finally, it should be noted that our study had a relatively low number of women and the ratio of men to women varied in each group. Although we observed no effect of gender on our findings, the limited number of women in our study does not allow us to be conclusive regarding possible gender differences/interactions with obesity and MetS influences on endothelial t-PA release.

Perspectives and Significance

The results of the present study indicate that the capacity of the endothelium to release t-PA is diminished to a similar extent in obese adults with and without the MetS. Exaggerated endothelial fibrinolytic dysfunction does not appear to be either a primary feature of the MetS or an underlying mechanism for the heightened atherothrombotic risk associated with obesity combined with the MetS compared with obesity alone.

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