Microvascular function in younger adults with obesity and metabolic syndrome: role of oxidative stress

Jacqueline K. Limberg,1 John W. Harrell,1 Rebecca E. Johansson,1 Marlowe W. Eldridge,1,2,3 Lester T. Proctor,4 Joshua J. Sebranek,4 and William G. Schrage1

1Department of Kinesiology, School of Education, University of Wisconsin, Madison, Wisconsin; 2The John Rankin Laboratory of Pulmonary Medicine, Department of Population Health Sciences, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin; 3Department of Pediatrics, University of Wisconsin Hospital and Clinics, Madison, Wisconsin; and 4Department of Anesthesiology, University of Wisconsin Hospital and Clinics, Madison, Wisconsin

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Am J Physiol Heart Circ Physiol 305: H1230–H1237, 2013. First published August 9, 2013; doi:10.1152/ajpheart.00291.2013.—Older adults with cardiovascular disease exhibit microvascular dysfunction and increased levels of reactive oxygen species (ROS). We hypothesized that microvascular impairments begin early in the disease process and can be improved by scavenging ROS. Forearm blood flow (Doppler ultrasound) was measured in 45 young (32 ± 2 yr old) adults (n = 15/group) classified as lean, obese, and metabolic syndrome (MetSyn). Vasodilation in response to endothelial (ACh) and vascular smooth muscle [nitroprusside (NTP) and epoprostenol (Epo)] agonists was tested before and after intra-arterial infusion of ascorbic acid to scavenge ROS. Vasodilation was assessed as a rise in relative vascular conductance (ml·min−1·100 mmHg−1). ACh and NTP responses were preserved (P = 0.825 and P = 0.924, respectively), whereas Epo responses were lower in obese and MetSyn adults (P < 0.05) than in lean controls. Scavenging of ROS via infusion of ascorbic acid resulted in an increase in ACh-mediated (P < 0.001) and NTP-mediated (P < 0.001) relative vascular conductance across all groups, suggesting that oxidative stress influences vascular responsiveness in adults with and without overt cardiovascular disease risk. Ascorbic acid had no effect on Epo-mediated vasodilation (P = 0.267). These results suggest that obese and MetSyn adults exhibit preserved endothelium-dependent vasodilation with reduced dependence on prostacyclin and are consistent with an upregulation of compensatory vascular control mechanisms.

VASCULAR DYSFUNCTION IS CONSIDERED an early event in atherogenesis and precedes the development of detectable cardiovascular disease (6, 20). However, few studies have assessed microvascular function in young obese adults and adults at increased cardiovascular disease risk [metabolic syndrome (MetSyn)] prior to manifestation of overt cardiovascular disease. Whereas several studies in obese adults support the presence of endothelial dysfunction in the conduit vasculature (1, 22, 38), the microcirculation is rarely examined. This is an important physiological distinction, given that conduit endothelial function does not correlate to the microcirculation (9, 14, 23). Taken together, microvascular function during the stages leading up to cardiovascular disease in humans is largely unexplored.

In animal models of obesity and MetSyn [obese Zucker rat (OZR)], microvascular dysfunction has been observed in the form of attenuated release and blunted responsiveness to endothelium-derived relaxing factors [i.e., prostacyclin (PGI2) and nitric oxide (NO)]. This may be due to reduced sensitivity of vessels to relaxing factors or reduced bioavailability. Reduced bioavailability may be due to increased oxidative stress and free radical scavenging, commonly observed in obesity-related disorders (2, 11, 45). Consistent with this concept, older obese adults have been shown to exhibit reduced endothelial function, which can be improved by reducing reactive oxygen species (ROS) with acute ascorbic acid infusion (28).

The impact of ROS on skeletal muscle microvascular function in younger obese adults and/or adults at increased cardiovascular disease risk (MetSyn) has not been systematically addressed, but it is reasonable to propose that excessive ROS might limit vasodilation. To provide opportunities for early prevention and treatment of vascular disease before overt microvascular dysfunction in middle age, it is essential to determine the onset and severity of early vascular decline and the mechanisms responsible. The current study sought to address these important questions by examining microvascular function using multiple pharmacological approaches to explore traditional vasodilatory pathways in younger adults along the cardiovascular disease continuum. We hypothesized that endothelial and smooth muscle function would be impaired in younger obese adults and/or adults with MetSyn compared with healthy lean controls. Given the increased levels of oxidative stress in obesity and further rise with MetSyn (13), we hypothesized that acute ROS scavenging would have a graded effect on vasodilatory responses to intra-arterial drug infusions, with the greatest improvement in MetSyn adults.

METHODS AND EXPERIMENTAL PROCEDURES

Subjects. Forty-five subjects were divided equally into three groups: lean, obese, and MetSyn (n = 15/group). Subjects were generally healthy, nonsmokers, and physically inactive (regular aerobic exercise <3 h/wk) and were not taking cardiovascular medications, as determined by self-report. Obese subjects had a body mass index (BMI) ≥30 kg/m2 but were otherwise healthy. Adults were characterized as MetSyn if they met three of the following National Cholesterol Education Program Adult Treatment Panel III criteria as modified by the American Diabetes Association: central obesity [waist circumference >88 cm (women) or >102 cm (men)], prehypertension (resting blood pressure ≥130/85 mmHg), hypertriglyceridemia (triglycerides ≥150 mg/dl), hyperglycemia (fasting glucose ≥100 mg/dl), and dyslipidemia (total cholesterol ≥200 mg/dl).
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≥100 mg/dl, and/or dyslipidemia [HDL <50 mg/dl (women) or <40 mg/dl (men)] (15). Of the adults with MetSyn, 15 met the criterion for waist circumference, 12 for blood pressure, 13 for HDL, 9 for triglycerides, and 6 for glucose. Obese adults and healthy controls did not meet the criterion for MetSyn.

Female subjects were not pregnant and were studied during the early follicular phase (days 1–5) of the menstrual cycle to avoid potential confounding effects of female hormone levels. Hormonal contraception was allowed [n = 2 (lean) and 1 (MetSyn)], and women on contraception were studied during the placebo phase. Subjects were instructed to refrain from exercise, nonsteroidal anti-inflammatory drugs, alcohol, and caffeine for 24 h prior to the study day.

Written informed consent was obtained from all subjects. All procedures were approved by the Institutional Review Board at the University of Wisconsin Madison and conformed to the standards set by the Declaration of Helsinki.

Measurements. Weight and height were measured, and body composition was determined by BMI (kg/m²), dual-energy X-ray absorptiometry (GE Lunar Prodigy, Milwaukee, WI), and waist circumference. Forearm volume (FHV) was determined using water displacement (21).

Arterial blood was collected after a 10-h fast, and triglyceride, HDL, and glucose levels were measured immediately (CardioChek, PTS Panels, Indianapolis, IN). Additional plasma samples were frozen at −80°C and analyzed at a later date for insulin (Millipore, Billerica, MA), C-reactive protein (CRP; R & D Systems, Minneapolis, MN), thiobarbituric acid-reactive substances (TBARS; Cayman Chemical, Ann Arbor, MI), and total antioxidant capacity (29).

Brachial artery catheterization. Under aseptic conditions and after local anesthesia (2% lidocaine), a 20-gauge, 5-cm catheter was placed in the brachial artery of the nondominant forearm in the antecubital fossa, with the subject in the supine position (the dominant arm was studied in 1 lean, 1 obese, and 1 MetSyn subject because of variations in the branching pattern of the brachial artery that precluded study with Doppler ultrasound). The catheter was used for continuous blood pressure measurement, local administration of vasoactive drugs, and blood sampling. The catheter was continuously flushed at 3 ml/h with heparinized saline.

Blood flow. Forearm blood flow (FBF; artery diameter and blood velocity) was measured using Doppler ultrasound (Vivid 7, General Electric, Milwaukee, WI). A 12-MHz linear array probe was placed approximately midway between the antecubital and axillary regions, medial to the biceps brachii muscle and proximal to the arterial catheter. The ultrasound probe operator adjusted the probe position to maintain a fixed intersection angle of ≤60°, with the sample volume adjusted to cover the width of the brachial artery (21). A mark was made on the skin over the brachial artery to ensure that measurements were taken in the same anatomic position for each trial.

Intra-arterial drug infusions. ACh (Novartis Pharmaceuticals, East Hanover, NJ), nitroprusside (NTP; Hospira, Lake Forest, IL), epoprostenol (Epo; GlaxoSmithKline, Research Triangle Park, NC), and ascorbic acid (Bioniche Pharma USA, Lake Forest, IL) were infused via the brachial artery catheter and were mixed specifically for each subject. A high dose of ascorbic acid was administered over 10 min; then a continuous maintenance dose of ascorbic acid was given for the remainder of the experiment. A 10-min washout period followed ACh and NTP trials; 20-min rest periods after Epo trials ensured return of hemodynamics to baseline levels (19, 25).

Data acquisition and analysis. Beat-to-beat heart rate (3-lead ECG; Datex-Ohmeda, Helsinki, Finland), blood pressure, and brachial artery blood velocity measurements were obtained throughout each trial. FBF was determined as the product of mean blood velocity (MBV, cm/s) and vessel cross-sectional area (CSA, radius in cm²) and was reported in ml/min [FBF = MBV × CSA (60 s/min)]. Pulse-wave velocities were measured beat-to-beat during the last 30 s of rest and each drug dose. Diameter measurements were taken immediately before increasing dosages and typically resulted in loss of pulse wave signal for 15 s. To determine vessel CSA, artery diameters were taken as the median of five measurements in late diastole. Artery diameter was measured on B-mode images in the part of the artery running perpendicular to the ultrasound beam and was identified by strong wall signals in the longitudinal section of the artery in each image. All measurements were assessed offline.

A commercial interface unit (Multigons Industries, Yonkers, NY) processed the angle-corrected, intensity-weighted Doppler audio information from the GE Vivid ultrasound system into a flow velocity signal that was sampled in real time with signal-processing software (PowerLab, ADInstruments, Colorado Springs, CO) (5, 19, 21, 31). All hemodynamic data were digitized, stored on a computer at 400 Hz, and analyzed offline using PowerLab; postprocessing using PowerLab’s Chart5 application package yielded MBV, blood pressure, and heart rate data.

The primary analysis was carried out to test whether vasoconstrictor responses to ACh, NTP, and/or Epo were reduced in human obesity and MetSyn compared with lean controls. The secondary analysis was carried out to test whether ascorbic acid infusion altered vasoconstrictor responses to ACh, NTP, and/or Epo differently between groups. To account for group differences in forearm size and resting blood pressure (Table 1), the main dependent variable was relative forearm vascular conductance (rFVC) [FBF measurements (ml/min) normalized for blood pressure and forearm size (ml-min-1·100 μmHg-1·dl-1)]. Because of differences in resting rFVC between groups and conditions, a change in rFVC was assessed [ΔrFVC = rFVCcondition − rFVCrest]. Taking into consideration previous research in older obese adults showing blunted ACh-mediated vasodilation that was improved with ascorbic acid (28), we determined a priori that n = 12 per group would be necessary to provide 80% power to detect a 9-unit difference in FBF at an α level of 0.05 across all groups and conditions.

Statistical analysis was done using SigmaPlot version 12.0 (Systat Software). Subject characteristics were compared using a one-way ANOVA to determine the significant effect of group (lean, obese, and MetSyn). Hemodynamic variables were analyzed using a three-way ANOVA to determine the significance of the effect of group, dose (baseline, low, medium, and high), and ascorbic acid on parameters of interest. Benferroni’s post hoc comparisons were performed when significant effects were observed. Pearson’s product-moment correlations were used to determine the association between peak vascular responses prior to ascorbic acid infusion and measures of cardiovascular disease risk (age, weight, waist circumference, body fat percentage, plasma glucose, HDL cholesterol, triglycerides, TBARS, CRP, and insulin). All data are presented as means ± SE, and significance was determined a priori at P < 0.05.
Table 1. Subject demographics

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<th>Lean (n = 14)</th>
<th>Obese (n = 15)</th>
<th>MetSyn (n = 15)</th>
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</thead>
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<tr>
<td>Sex, M/F</td>
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<td>11/4</td>
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<td>Height, cm</td>
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<td>Weight, kg</td>
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<td>Waist, cm</td>
<td>79 ± 3</td>
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<td>119 ± 4*</td>
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<td>Body fat, %</td>
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<td>40 ± 2*</td>
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<td>FA¥, ml</td>
<td>1045 ± 51</td>
<td>1352 ± 59*</td>
<td>1435 ± 98*</td>
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<td>MAB¥, mmHg</td>
<td>90 ± 2</td>
<td>97 ± 3</td>
<td>105 ± 2*</td>
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<td>74 ± 2</td>
<td>90 ± 5*</td>
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<tr>
<td>Insulin, µU/ml</td>
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<td>40 ± 8*</td>
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<td>Triglyceride, mg/dl</td>
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<td>90 ± 9</td>
<td>183 ± 26*†</td>
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<tr>
<td>HDL, mg/dl</td>
<td>46 ± 4</td>
<td>34 ± 5</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>CRP, mg/dl</td>
<td>0.4 ± 0.1</td>
<td>1.5 ± 0.4</td>
<td>2.0 ± 0.5*</td>
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<tr>
<td>TBARS, MDA units</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>1.9 ± 0.3</td>
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<tr>
<td>Physical activity, kcal/wk</td>
<td>2,354 ± 523</td>
<td>2,081 ± 530</td>
<td>2,039 ± 450</td>
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</tbody>
</table>

Values are means ± SE; n = 15 per group unless otherwise noted: waist (lean n = 14), body fat (obese n = 14), insulin (lean n = 11, obese n = 12, MetSyn n = 12), HDL (obese n = 14), C-reactive protein (CRP; lean n = 14), thioarbituric acid-reactive substances (TBARS; lean n = 14), physical activity (MetSyn n = 13). M, male; F, female; BMI, body mass index; FA¥, forearm volume; MAB¥, mean arterial blood pressure; MDA, malondialdehyde. Effect of group: *P < 0.05 vs. lean, †P < 0.05 vs. obese.

RESULTS

Subject characteristics. Fifteen adults with MetSyn, 15 obese adults, and 15 lean healthy adults completed the study (83% white non-Hispanic, 3% white Hispanic, 8% Asian American, and 6% African American). Subject characteristics are summarized in Table 1. There were no significant differences in age, HDL cholesterol, TBARS, or physical activity between groups (P > 0.05). Subjects in the obese and MetSyn groups were clinically obese, displaying significantly higher weight, BMI, waist circumference, and percent body fat (P < 0.05) than lean adults. Obese and MetSyn subjects also had greater FA¥ (P < 0.05). Glucose, insulin, triglycerides, resting mean blood pressure, and CRP were not significantly different between lean and obese adults (P > 0.05). Adults with MetSyn exhibited higher fasting glucose, insulin, triglycerides, resting mean blood pressure, and CRP than lean and/or obese adults (P < 0.05). Intra-arterial infusion of ascorbic acid resulted in a significant increase in plasma total antioxidant capacity (2.6 ± 0.4 to 6.4 ± 0.7 arbitrary units, an increase of 196 ± 35%, P < 0.01), establishing the efficacy of ascorbic acid infusion.

Systemic hemodynamics during infusions. Brachial artery diameter, heart rate, and blood pressure were greater in MetSyn and obese adults than in healthy controls (P < 0.05). Brachial artery diameter and heart rate did not change with ascorbic acid or drug infusion in any group (P > 0.05). Ascorbic acid infusion resulted in an increase in blood pressure during ACh and NTP trials (P < 0.05) but had no effect on blood pressure with Epo infusion (P > 0.05); these responses were not different between groups (data not shown).

Endothelium-dependent vasodilation. Relative FBF (rFBF) and rFVC increased with increasing dose of ACh (Table 2). The increase in rFVC with ACh infusion was not different between groups (Fig. 1A). Ascorbic acid increased rFBF and rFVC responses to ACh (Table 2, Fig. 2); however, the effect was not specific to group.

Smooth muscle responsiveness to NO. rFBF and rFVC increased with increasing dose of NTP (Table 3). The increase in rFVC with NTP infusion was not different between groups (Fig. 1B). Ascorbic acid increased rFBF and rFVC responses to NTP (Table 3, Fig. 3). The effect of ascorbic acid on NTP-mediated vasodilation tended to be group-specific (group-ascorbic acid interaction, P = 0.051), with a trend for no effect of ascorbic acid on NTP responses in obese adults (Fig. 3B).

Smooth muscle responsiveness to PGI². rFBF and rFVC increased with increasing dose of Epo (Table 4). The increase in rFVC with Epo infusion was blunted in obese and MetSyn adults compared with healthy lean controls (Fig. 1C). Ascorbic acid appeared to have no effect on rFBF or rFVC in any group (Table 4, Fig. 4).

Relationships between peak responsiveness and risk factors. To explore potential contributors to altered vascular control, relationships between cardiovascular disease risk factors and endothelial and vascular smooth muscle peak responses were assessed prior to infusion of ascorbic acid. Significant relationships were observed between TBARS and peak responses to ACh (ρ = −0.37, P = 0.01) and NTP (ρ = −0.31, P = 0.04), such that higher levels of oxidative stress were associated with lower vasodilation. Similar relationships were not observed.

Table 2. Responses to ACh infusion

<table>
<thead>
<tr>
<th></th>
<th>Lean (n = 14)</th>
<th>Obese (n = 15)</th>
<th>MetSyn (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-AA</td>
<td>Post-AA</td>
<td>Pre-AA</td>
</tr>
<tr>
<td>rFBF, ml.min⁻¹.dl⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8 ± 2</td>
<td>9 ± 2</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Low*</td>
<td>19 ± 4</td>
<td>26 ± 3</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Medium#</td>
<td>23 ± 4</td>
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<td>20 ± 3</td>
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<td>High#*</td>
<td>30 ± 5</td>
<td>36 ± 5</td>
<td>30 ± 4</td>
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<tr>
<td>AUC‡</td>
<td>489 ± 90</td>
<td>649 ± 133</td>
<td>478 ± 81</td>
</tr>
<tr>
<td>rFVC, mmHg.min⁻¹.100 ml.min⁻¹.dl⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>9 ± 2</td>
<td>9 ± 2</td>
<td>7 ± 1</td>
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<td>Low*</td>
<td>20 ± 4</td>
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<td>20 ± 3</td>
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<tr>
<td>High#*</td>
<td>31 ± 5</td>
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<td>29 ± 4</td>
</tr>
<tr>
<td>AUC‡</td>
<td>499 ± 99</td>
<td>670 ± 148</td>
<td>463 ± 77</td>
</tr>
</tbody>
</table>

Values are means ± SE; rFBF, relative forearm blood flow; rFVC, relative forearm vascular conductance; AA, ascorbic acid; AUC, area under the curve. Effect of group: *P < 0.05 vs. lean, †P < 0.05 vs. obese. Effect of dose: ‡P < 0.05 vs. baseline, bP < 0.05 vs. low, cP < 0.05 vs. medium. Effect of AA: ‡P < 0.05 vs. pre-AA.
with Epo \((r = -0.27, P = 0.09)\). No other significant relationships were observed.

**DISCUSSION**

This study directly examined endothelial and smooth muscle function before and after ROS scavenging in young obese adults and adults with MetSyn. Novel findings from this study show that endothelial function is preserved in young (32 ± 2 yr old) obese and MetSyn adults, despite impaired PGI₂-mediated vasodilation. While they are speculative, these results suggest that obese and MetSyn adults rely on compensatory vascular control mechanisms to overcome depressed PGI₂ signaling. Given that ROS scavenging had no effect on impairments in PGI₂-mediated vasodilation, vascular smooth muscle responses to PGI₂ appear to be independent of ROS signaling. In contrast, ROS scavenging resulted in an increase in vascular responsiveness to ACh and NTP across all groups (i.e., not limited to obese and MetSyn adults). These unexpected findings suggest that oxidative stress can influence vascular responsivity in sedentary young adults with and without overt cardiovascular disease risk. Taken together, early changes in vascular smooth muscle function in young, relatively healthy obese and MetSyn adults suggest that compensatory mechanisms contributing to preserved vascular function occur early in the disease process.

**Vascular function in obesity and MetSyn.** Animal research supports endothelial dysfunction in MetSyn (reviewed in Ref. 10), and blunted vascular responsiveness to ACh in older overweight and obese adults was observed in previous studies in humans (7, 36, 41, 44). Therefore, we hypothesized that ACh-mediated vasodilation would be blunted in younger obese and MetSyn humans; in contrast, endothelial function was preserved (Fig. 1A). In support of our findings, Nielsen et al. (26) showed that endothelium-dependent vasodilation was similar in younger (~35 yr old) obese and nonobese men; Villela and colleagues (43) found that when subjects >40 yr of age were excluded from their analysis, the inverse relationship between BMI and ACh responses was abolished. Thus our

![Figure 1](http://ajpheart.physiology.org/)

**Fig. 1.** Increase in relative forearm vascular conductance (rFVC) from baseline with intra-arterial infusions. **A:** rFVC increased with increasing dose of ACh (main effect of dose, \(P < 0.001\)), and responses were not different between groups (group-dose interaction, \(P = 0.677\)). **B:** rFVC increased with increasing dose of nitroprusside (NTP; main effect of dose, \(P < 0.001\)), and responses were not different between groups (group-dose interaction, \(P = 0.638\)). **C:** rFVC increased with increasing dose of epoprostenol (Epo; main effect of dose, \(P < 0.001\)), and responses were greater in lean adults than in adults with metabolic syndrome (MetSyn) and obese adults (group-dose interaction, \(P = 0.049\)). Post hoc comparisons indicate differences at the following Epo doses: medium [lean vs. obese \((P = 0.022)\) and high [lean vs. obese \((P < 0.001)\) and lean vs. MetSyn \((P = 0.014)\)]. *\(P < 0.05\) (group-dose interaction).

![Figure 2](http://ajpheart.physiology.org/)

**Fig. 2.** Change in rFVC response to ACh infusion with ascorbic acid (AA) infusion in lean \((n = 14; A)\), obese \((n = 15; B)\), and MetSyn \((n = 15; C)\) groups. rFVC increased with ascorbic acid infusion (main effect of AA, \(P < 0.001\)); however, effect of AA on ACh-mediated vasodilation was not different between groups (group-AA interaction, \(P = 0.474\)). ‡\(P < 0.05\) vs. pre-AA (main effect of AA).
The lack of group differences in ACh-mediated vasodilation (Fig. 1A).

Whereas the integrated vascular response to ACh remained intact, obese adults and adults with MetSyn exhibited blunted responsiveness to the PGI2 analog Epo (Fig. 1C). PGI2-mediated vasodilation in the peripheral circulation was previously unexamined in human obesity and MetSyn; however, findings are consistent with impaired vasodilation to PGI2 analogs (12, 46) and reduced PGI2 synthesis (16) previously observed in the OZR. PGI2 (IP) receptors activate potassium channels, leading to membrane hyperpolarization and vasodilation (32, 35). Thus downregulation of the IP receptors and/or reduced ATP-sensitive potassium (K\textsubscript{ATP}) channel-mediated vasodilation could contribute to the impairment in PGI2-mediated vasodilation in the current study. In support of this idea, the OZR has been shown to exhibit decreased K\textsubscript{ATP} channel sensitivity (17) and a trend for lower IP receptor expression (16). Together, the present results from humans appear to confirm observations in animal models and add to the growing body of research supporting the presence of smooth muscle adaptations in younger obese and MetSyn humans.

In contrast to reductions in PGI2-mediated vasodilation, smooth muscle responsiveness to the NO donor NTP was
preserved in human obesity and MetSyn (Fig. 1B). Similar results were observed previously in the microcirculation (7, 26, 28, 41, 42, 44). However, it is important to acknowledge recent work identifying impairments in smooth muscle responsiveness to NO in the conduit (brachial) arteries of obese adults (4), suggesting that the impact of obesity on vascular smooth muscle may vary by anatomic location.

Taken together, endothelium-dependent vasodilation is preserved in younger obese adults and adults with MetSyn (Fig. 1A). Given the impairments in smooth muscle responsiveness to PGI2 (Fig. 1C), these results suggest that the primary mechanisms behind maintained ACh responses are not PGI2-dependent at the level of the smooth muscle. This finding is consistent with an upregulation of alternative vascular control mechanisms. Along these lines, hypertensive rats have been shown to exhibit normal ACh-mediated dilation through primarily NO-independent mechanisms (37), and arteries from cholesterol-fed rabbits compensate for impaired NO production via an increase in calcium-dependent potassium (BKCa) channel activity (24). We speculate that obese adults and adults with MetSyn exhibit preserved endothelium-mediated vasodilation early in the disease process as a result of altered vascular control mechanisms. However, as the syndrome progresses, such adaptations may be unable to compensate, resulting in microvascular dysfunction and clinical manifestation of cardiovascular disease. These results are just the first step, and future studies are necessary to directly test these proposed complicated interactions using specific endothelial antagonists (e.g., N\textsuperscript{G}-monomethyl-L-arginine and indomethacin).

Role of oxidative stress in obesity and MetSyn. To explore potential contributors to altered vascular control, we examined the relationship between the peak response to intra-arterial infusions of ACh, NTP, and Epo and cardiovascular disease risk factors across groups. Interestingly, the only factor to consistently and significantly impact vascular responses was plasma TBARS. TBARS is a by-product of lipid peroxidation and, thus, is a measure of the damage produced by oxidative stress (3). Consistent with our initial hypothesis, adults with the lowest peak response to endothelial and vascular smooth muscle stimulation were also those exhibiting the greatest level of oxidative stress.

Along similar lines, older obese adults have been shown to exhibit reduced endothelial function that can be improved by reducing ROS with acute ascorbic acid infusion (28). However, on the basis of relatively intact vascular responses to ACh in young obese adults and adults with MetSyn compared with lean controls (Fig. 1A), we were surprised to observe significant increases in ACh-mediated vasodilation after ascorbic acid infusion in obese (Fig. 1B), similar to what was observed (baseline vs. ascorbic acid infusion). These results are unexpected and suggest that the impaired ACh response in obese adults and adults with MetSyn may be unable to compensate, owing to persistent oxidative stress.

**Table 4. Responses to epoprostenol infusion**

<table>
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<td>rFVC, ml·min(^{-1})·dl(^{-1})</td>
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<td>9 ± 2</td>
<td>9 ± 1</td>
<td>7 ± 1(^*)</td>
</tr>
<tr>
<td>Low(^a)</td>
<td>18 ± 4</td>
<td>17 ± 2</td>
<td>15 ± 3(^*)</td>
</tr>
<tr>
<td>Medium(^a,b)</td>
<td>27 ± 6</td>
<td>28 ± 4</td>
<td>18 ± 4(^*)</td>
</tr>
<tr>
<td>High(^a,b,c)</td>
<td>38 ± 11</td>
<td>34 ± 5</td>
<td>21 ± 4(^*)</td>
</tr>
<tr>
<td>AUC</td>
<td>151 ± 40</td>
<td>145 ± 25</td>
<td>87 ± 20</td>
</tr>
</tbody>
</table>

Values are means ± SE. Main effect of group: \(^*P < 0.05\) vs. lean, \(^†P < 0.05\) vs. obese. Main effect of dose: \(^*P < 0.05\) vs. baseline, \(^bP < 0.05\) vs. low, \(^cP < 0.05\) vs. medium.

\(A\) and \(B\) show the change in rFVC response to Epo infusion with ascorbic acid infusion in lean \((A; n = 13)\), obese \((B; n = 14)\), and MetSyn \((C; n = 14)\) groups. rFVC increased with increasing dose (main effect of dose, \(P < 0.001\)). There was no effect of AA infusion in any group (main effect of AA, \(P = 0.267\); group-AA interaction, \(P = 0.831\)).
acid infusion (Fig. 2). Similarly, NTP-mediated vasodilation increased after ascorbic acid infusion (Fig. 3). Given that ascorbic acid can scavenge free radicals and increase NO bioavailability (27), these results suggest that improvements in vasodilatory responses may be the result of ROS-mediated reductions in NO bioavailability at baseline. However, it is important to note that the effect of ascorbic acid on NTP-mediated vasodilation tended to be group-specific (group-ascorbic acid interaction, \( P = 0.051 \)), with a trend for no effect of ascorbic acid on NTP responses in obese adults (Fig. 3B). Interestingly, improvements in ACh- and NTP-mediated vasodilation with ascorbic acid infusion were also observed in lean control subjects (Figs. 2 and 3). Considering that all subjects in the current study were matched for low levels of physical activity, we speculate that sedentary behaviors across all groups may have contributed to the lack of group differences in ACh and NTP responses (Fig. 1A) in addition to the group-independent improvements in ACh- and NTP-mediated vascular responses with ascorbic acid infusion (Figs. 2 and 3). These results suggest that oxidative stress can influence vascular responsiveness in physically inactive young adults without overt cardiovascular disease and are consistent with the idea that physical activity lowers disease risk (33, 34).

In contrast, impairments in PGI2-mediated vasodilation in obese and MetSyn adults were not improved with ascorbic acid infusion (Fig. 4B), suggesting that early impairments in vascular smooth muscle responses to PGI2 are ROS-independent. In support of this idea, impairments in PGI2-mediated vasodilation in the OZR appear to be the result of impaired PGI2 release and/or decreased prostaglandin receptor expression (16).

Experimental considerations. Because our subjects were relatively young, we did not observe large group differences in markers of inflammation and oxidative stress, although CRP levels were significantly higher in adults with MetSyn (Table 1). One possible explanation may be that local, physiologically relevant levels of ROS are not detectable with methods used in the current study. Despite this potential limitation, linear relationships were observed between TBARS and vascular responses, such that those adults with increased oxidative stress exhibited blunted vasodilation. In addition, we were able to increase antioxidant capacity substantially in all groups with ascorbic acid infusion and observed functional changes in local, microvascular responses (Figs. 2 and 3). Furthermore, conclusions were consistent across multiple methods of data expression (rFBF, rFVC, rFVC area under the curve, and ΔrFVC; Tables 2–4, Figs. 2–4), providing confidence in the present conclusions. Taken together, even low levels of oxidative stress appear to contribute to altered vascular control.

A strength of the current study was the use of multiple pharmacological agents to explore traditional vasodilatory pathways. Current results preserved endothelial function in human obesity and MetSyn using the endothelial agonist ACh. Given that previous studies showed consistent responses to agonist-stimulated endothelium-dependent vasodilation across several agonists (e.g., ACh, methacholine, bradykinin, substance P, and isoproterenol) (42), the use of ACh in the present study does not limit the current conclusions. However, future studies should consider examining the contribution of NO, PGI2, and/or other compensatory pathways to agonist-mediated responses via the use of specific inhibitors (e.g., N ω- monomethyl-L-arginine and indomethacin) (31). Such methods would strengthen our understanding of vascular adaptations in human obesity and MetSyn and provide more extensive mechanistic evidence for upregulation of specific compensatory vascular control mechanisms, which may compensate for reduced PGI2-mediated vasodilation.

Conclusion. Vascular dysfunction is considered an early event in atherogenesis and precedes the development of detectable cardiovascular disease. However, very little is known about the onset, progression, and potential compensatory adaptations in obesity-related conditions prior to middle age and the development of overt cardiovascular dysfunction. In the current study, younger obese adults and adults with MetSyn demonstrated preserved integrative responses to the endothelium-dependent vasodilator ACh. However, the primary mechanisms behind maintained ACh responses are likely less reliant on PGI2, given that smooth muscle responses to PGI2 were blunted in obese and MetSyn adults. We propose that such vascular adaptations are early signs of dysfunction that may require compensatory mechanisms during early cardiovascular disease to preserve overall responses. Interestingly, impairments in PGI2-mediated vasodilation are ROS-independent, despite present results suggesting that oxidative stress can influence vascular control in physically inactive young adults without overt cardiovascular disease. Together, these findings highlight the presence of potential subtle vascular changes that, with increasing age and/or duration of obesity, likely result in a clinical manifestation of cardiovascular disease.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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