**ENDOTHELIAL VASODILATOR FUNCTION IN NORMAL
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ENDOTHELIAL VASODILATOR FUNCTION IN NORMAL WEIGHT ADULTS WITH METABOLIC SYNDROME

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ABSTRACT

Metabolic Syndrome (MetS) typically presents with obesity; however, obesity is not a requisite characteristic for MetS classification and related vascular risk. We tested the hypothesis that MetS, independent of excess adiposity, is associated with impaired endothelial vasodilator dysfunction. Thirty-two sedentary, middle-aged adults were studied: 11 normal weight (9 M/2 F; BMI $24.0 \pm 0.3 \text{ kg/m}^2$); 11 normal weight with MetS (9 M/2 F; $24.7 \pm 0.3 \text{ kg/m}^2$); and 10 obese without MetS (8 M/2 F; $31.4 \pm 0.5 \text{ kg/m}^2$). MetS was established according to NCEP ATP III criteria. Forearm blood flow (FBF) responses to intra-arterial infusions of acetylcholine and sodium nitroprusside were measured via strain-gauge plethysmography. FBF responses to acetylcholine were $\sim 20\%$ lower ($P < 0.05$) in the normal weight with MetS (from 4.0 ± 0.3 to $13.0 \pm 1.0 \text{ mL/100 mL tissue/min}$) and obese (from 4.8 ± 0.2 to $12.2 \pm 1.1 \text{ mL/100 mL tissue/min}$) compared with normal weight (from 4.6 ± 0.4 to $15.8 \pm 0.7 \text{ mL/100 mL tissue/min}$) subjects. Of note, FBF responses to acetylcholine were similar between the normal weight with MetS and obese adults. There were no differences in FBF response to sodium nitroprusside between groups. These data indicate that the presence of MetS, independent of obesity, is associated with diminished endothelium-dependent vasodilation. Endothelial vasodilator dysfunction may underlie the increased cardiovascular risk in normal weight adults with MetS.

Keywords: endothelium, vasodilation, forearm blood flow, metabolic syndrome, normal weight

INTRODUCTION

Metabolic Syndrome (MetS) refers to a clustering of risk factors including dyslipidemia, prehypertension, hyperglycemia, and central adiposity that appear together and ultimately accelerate the development of atherothrombotic cardiovascular disease (CVD) (Grundy 2002; Ingelsson et al. 2007). MetS typically presents with obesity; however, data from the National Health and Nutrition Examination Survey (NHANES) indicates that obesity is not a prerequisite for the development of MetS (Park et al. 2003; Ervin 2009). Nearly 10% of adults in the United States with a normal body mass index (BMI) are reported to have MetS (Ervin 2009). Importantly, normal weight adults with MetS are at an increased risk of CVD morbidity and mortality compared with metabolically healthy normal weight adults (Fan et al. 2013; Yoo et al. 2014). The mechanisms responsible for the elevated risk for CVD in normal weight adults with MetS are not completely understood, but likely extend beyond the constellation of traditional risk factors comprising MetS (Du et al. 2015; Yoo et al. 2015).

Impaired endothelium-dependent vasodilation, a hallmark characteristic of endothelial dysfunction, is an underlying factor in the pathogenesis of CVD (Quyyumi 1998; Campia et al. 2012). Endothelium-dependent vasodilation is markedly impaired in obese adults with MetS compared with metabolically healthy obese adults (Schinzari et al. 2015). This finding underscores the role of MetS, above and beyond that of excess adiposity alone, in endothelial vasodilator dysfunction. However, the influence of MetS, independent of obesity, on endothelium-dependent vasodilation is currently unknown. Endothelial vasodilator dysfunction may be a contributing factor to the increased CVD risk in normal weight adults with MetS.

Accordingly, we tested the hypothesis that endothelium dependent vasodilation is impaired in normal weight adults with MetS compared with normal weight adults without MetS. To address this aim, we used an isolated forearm model to assess endothelial vasodilator function in metabolically healthy normal weight adults and normal weight adults with MetS.

METHODS AND PROCEDURES

Subjects. Thirty-two sedentary, middle-aged adults were studied: 11 normal weight without MetS (age 55 ± 2 yr; 9 M/2 F); 11 normal weight with MetS (56 ± 3 yr; 9 M/2 F); and 10 obese without MetS (56 ± 2 yr; 8 M/2 F). All normal weight adults had a BMI < 26.0 kg/m² whereas all obese adults had a BMI ≥ 30.0 kg/m². MetS was established according to the National Cholesterol Education Program (NCEP) Adult Treatment Plan (ATP) III criteria (Expert Panel on Detection and Treatment of High Blood Cholesterol in 2001). All subjects were nonsmokers, non-medicated (including vitamins), and free of overt CVD as assessed by medical history, physical examination, resting and exercise electrocardiograms, and fasting blood chemistries. Women were at least one year postmenopausal and had never taken or discontinued hormone replacement therapy at least one year prior to study start. The Institutional Review Board at the University of Colorado, Boulder, approved the study. All subjects provided written informed consent according to University guidelines.

Body Composition. Body mass was measured to the nearest 0.1 kilogram using a medical beam balance (Detecto, Webb City, MO) and height was measured to the nearest centimeter. BMI was calculated as weight (kilograms) divided by height (meters) squared. Minimal waist circumference was measured according to published guidelines

(Lohman et al. 1988). Percent body fat was determined by dual-energy X-ray absorptiometry (Lunar Radiation, Madison, WI).

Metabolic Measures. Fasting plasma lipid, lipoprotein, glucose, and insulin concentrations were determined using standard techniques (Van Guilder et al. 2008a) by the clinical laboratory at the Clinical and Translational Research Center at the University of Colorado, Boulder. Insulin resistance was estimated using the homeostasis model of assessment (HOMA-IR) (Matthews et al. 1985).

Intra-arterial Infusion Protocol. All measurements were performed in a temperature-controlled room between 7 and 10 AM after a 12-hour overnight fast as previously described (Hoetzer et al. 2003). Briefly, a 5-cm, 20-gauge catheter was inserted into the brachial artery of the non-dominant arm under local anesthesia (1% lidocaine). Forearm blood flow (FBF) was measured using strain-gauge venous occlusion plethysmography (D.E. Hokanson, Bellevue, WA). Following the measurement of resting blood flow for five minutes, acetylcholine was infused intra-arterially at rates of 4.0, 8.0 and 16.0 $\mu\text{g}/100\text{ mL tissue}/\text{min}$ and sodium nitroprusside at 1.0, 2.0 and 4.0 $\mu\text{g}/100\text{ mL tissue}/\text{min}$ for five minutes at each dose.

Statistical Analysis. Differences in subject characteristics and area under the curve data were determined by one-way analysis of variance (ANOVA). Group differences in the FBF responses to each vasoactive drug were determined by repeated measures ANOVA. Post hoc testing using Newman-Keuls method was performed to determine within group differences at each concentration. Simple and forward stepwise multiple regression analyses were used to determine the relations between the outcome variables and variables of interest. Although the number of women in the study was

small, their values were almost identical to the men. As such, the data were pooled and presented together. All values are expressed as mean $\pm$ SEM. Statistical significance was set *a priori* at $P < 0.05$.

RESULTS

Select subject characteristics are presented in the Table. Body mass, BMI, percent body fat, and waist circumference were higher in the obese compared with the normal weight and normal weight with MetS groups. MetS component variables were significantly higher in the normal weight with MetS compared with the normal weight group, specifically: systolic blood pressure, triglycerides, and glucose, whereas HDL-cholesterol was significantly lower. Triglyceride and glucose concentrations were higher in the normal weight with MetS compared with the obese group. Insulin concentrations were significantly higher in the obese group compared with both normal weight groups, while HOMA-IR was significantly higher in the normal weight with MetS and obese groups compared with the normal weight group.

Figure 1 shows the FBF response to acetylcholine and sodium nitroprusside. FBF to acetylcholine was significantly blunted ($\sim 20\%$; $P < 0.05$) in the normal weight with MetS group (from 4.0 ± 0.3 mL/100 mL tissue/min to 13.0 ± 0.9 mL/100 mL tissue/min) compared with normal weight controls (from 4.6 ± 0.4 mL/100 mL tissue/min to 15.8 ± 0.7 mL/100 mL tissue/min). The FBF response to acetylcholine in the normal weight with MetS group was not significantly different from the obese group (from 4.8 ± 0.2 mL/100 mL tissue/min to 12.2 ± 1.1 mL/100 mL tissue/min). Total FBF to acetylcholine (area under the curve) was approximately 30% lower ($P < 0.05$) in the normal weight with MetS

(55.2 ± 8.4 mL/100 mL tissue) and obese (52.4 ± 7.9 mL/100 mL tissue) groups compared with the normal weight controls (78.5 ± 6.5 mL/100 mL tissue). There were no significant differences ($P=0.45$) in the vasodilator response to sodium nitroprusside between groups.

In the normal weight subjects (with and without MetS), peak FBF response to acetylcholine was inversely and significantly associated with fasting plasma glucose and systolic blood pressure (Figure 2). Stepwise regression analysis revealed that fasting plasma glucose was the primary determinant of the vasodilator response to acetylcholine, accounting for 35% of the variability ($R^2=0.35$; $\beta=-0.162$). Aside from fasting plasma glucose and systolic blood pressure, there were no significant correlations between the vasodilation to acetylcholine and other anthropometric, hemodynamic or metabolic variables.

DISCUSSION

The primary new finding of the present study is that endothelium-dependent vasodilation is impaired in normal weight adults with MetS compared with normal weight adults without MetS. Moreover, the degree of impairment in endothelial vasodilator function in normal weight adults with MetS is similar to that of obese adults without MetS.

MetS confers significant increase in CVD risk. A meta-analysis involving nearly 1 million adults concluded that the presence of MetS is associated with a two-fold increased risk for CVD, myocardial infarction and stroke (Mottillo et al. 2010). Classically, MetS presents with excess adiposity (Despres and Lemieux 2006) exacerbating CVD risk with obesity (Grundy 2004). The mechanisms underlying the

increased CV risk with MetS are not fully understood, but endothelial dysfunction is considered a primary contributing factor (Suzuki et al. 2008). In obese adults with MetS, endothelial vasomotor function, a key feature of endovascular health, is negatively affected (Schinzari et al. 2010; Rocha et al. 2014; Schinzari et al. 2015). For example, Schinzari, et al. (Schinzari et al. 2015) demonstrated that endothelium-dependent vasodilator dysfunction is worse in obese adults with MetS than obese adults without MetS. Moreover, we recently demonstrated (Rocha et al. 2014) that MetS is associated with higher endothelin-1-mediated vasoconstrictor tone in obese adults. The results of the present study significantly extend these findings by demonstrating that endothelium-dependent vasodilation is markedly reduced in normal weight adults with MetS. Indeed, the forearm vasodilator response to the endothelial agonist acetylcholine was ~30% lower in the normal weight MetS group compared with the normal weight group without MetS. Thus, the negative influence of MetS on endothelial vasodilator capacity is not limited to obese adults. Reduction in endothelium-dependent vasodilation occurs early in atherogenesis before histological or angiographic evidence of disease (Yasue et al. 1990) and may thus contribute to MetS-related increased cardiovascular risk (Suzuki et al. 2008).

An interesting finding of the present study is that endothelial vasodilator function was almost identical between the normal weight group with MetS and the obese adults free of MetS despite marked anthropometric group differences in body mass, BMI, body fat percentage and waist circumference. Consistent with previous studies (Van Guilder et al. 2008b; Han et al. 2011), we demonstrate that endothelium-dependent vasodilation is significantly blunted in obese adults free of other cardiometabolic risk factors. The

presence of the MetS syndrome in normal weight adults appears to render an endovascular phenotype similar to obesity *per se*. It is interesting to note that although the normal weight/MetS subjects in the present study presented hemodynamic and metabolic characteristics that met MetS criteria, their respective laboratory values were not grossly abnormal and none of the subjects were hypertensive or diabetic. Thus, even a modest cardiometabolic MetS profile is associated with profound endothelial vasodilator dysfunction. Considering ~10% of normal weight adults in the United States have MetS (Ervin 2009), continued clinical vigilance for, and treatment of, MetS regardless of body composition status is important for CVD prevention especially in middle-aged and older adults.

The mechanisms underlying the impairment in endothelium-mediated vasodilation associated with the normal weight MetS phenotype are unclear. It is experimentally difficult in clinical studies to tease out whether the MetS-related dysfunction is due to an individual component of the syndrome or a constellation of factors. In the present study, systolic blood pressure and fasting glucose concentrations were inversely correlated with peak forearm blood flow to acetylcholine in normal weight groups, suggesting both as putative mediators underlying the differences in vasodilation. This notion is supported by previous studies demonstrating that elevations in systolic blood pressure and blood glucose concentrations, independently (Vehkavaara et al. 1999; Plavnik et al. 2007; Weil et al. 2011) and combined (Ghiadoni et al. 2008), negatively influence endothelium-dependent vasodilation. Although not measured herein, MetS has been shown to adversely influence nitric oxide bioavailability (Tesauro et al. 2005; Schinzari et al. 2013) and endothelin-1 mediated vasoconstriction in obese adults (Rocha

et al. 2014; Schinzari et al. 2015). It is possible that the MetS-related diminution in endothelium-dependent vasodilation in normal weight adults may be due to dysregulation of the nitric oxide and endothelin-1 systems. Considering that both blood pressure (Weil et al. 2011; Weil et al. 2012) and glucose concentrations (Ihlemann et al. 2003; Diehl et al. 2013) also independently affect nitric oxide bioavailability and endothelin-1 system activity, these mechanisms are certainly plausible and reasonable areas for future studies.

There are two important experimental considerations regarding this study that merit mention. Firstly, this study was cross-sectional in nature; thus, lifestyle and genetic factors may have influenced our results. In an effort to minimize the effects of lifestyle, all subjects were sedentary, nonsmokers who were not taking any medication including vitamin supplements that could influence endothelium-dependent vasodilation. Secondly, the small number of women in the study precludes us from definitively addressing possible gender interactions/differences with MetS-related endothelial vasodilator dysfunction. Relatedly, we acknowledge the modest overall sample size of the study. However, the employment of strict inclusion criteria to limit confounding variables and the robust differences in blood flow observed between the normal weight groups resulted in sufficient statistical power (determined post-hoc; 80% power; effect size 1.12) to assess group-differences with minimal risk of committing a type-II error.

CONCLUSION

In conclusion, the results of the present study demonstrate that the presence of MetS independent of excess adiposity is associated with diminished endothelium-dependent vasodilation. Endothelial vasodilator dysfunction may contribute to the

increased cardiovascular risk reported in normal weight adults with MetS.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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TABLES

Table. Selected subject characteristics

Variable	Normal Weight (n=11)	Normal Weight + MetS (n=11)	Obese (n=10)
Age (years)	55±2	56±3	56±2
Body mass (kg)	75.0±3.1	79.7±2.1	94.7±3.9*†
BMI (kg/m ²)	24.0±0.3	24.7±0.3	31.4±0.5*†
Body fat (%)	25.5±2.0	27.2±1.6	36.3±2.1*†
Waist circumference (cm)	85.6±2.8	89.6±2.4	105.3±2.6*†
Systolic BP (mmHg)	113±2	129±3*	128±2*
Diastolic BP (mmHg)	74±3	78±2	81±1*
Total cholesterol (mg/dL)	176.7±5.5	190.6±8.5	187.9±16.8
HDL cholesterol (mg/dL)	49.0±3.9	39.5±2.1*	47.6±2.1
LDL cholesterol (mg/dL)	110.3±6.9	115.3±6.8	121.6±14.4
Triglycerides (mg/dL)	87.5±7.2	195.5±21.0*	93.8±10.9†
Glucose (mg/dL)	89.5±2.1	103.6±4.3*	92.8±3.0†
Insulin (μU/L)	5.1±0.8	6.8±0.6	8.2±1.0*
HOMA-IR	1.1±0.2	1.7±0.2*	2.1±0.3*

BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model of insulin resistance.

Values are mean ± SEM.

*P<0.05 vs normal weight

†P<0.05 vs normal weight + MetS

FIGURE LEGENDS

Figure 1. FBF responses (panel A) and total FBF (area under the curve; panel B) to acetylcholine and FBF responses to sodium nitroprusside (panel C) in the normal weight adults, normal weight adults with MetS and obese groups. Values are mean $\pm$ SEM.

*P<0.05 vs. normal weight.

Figure 2. Correlations between peak FBF response to acetylcholine and glucose (panel A) and systolic blood pressure (panel B) in normal weight subjects (with and without MetS).

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