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BASAL ENDOTHELIAL NITRIC OXIDE RELEASE IS PRESERVED IN OVERWEIGHT AND OBESE ADULTS

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Abstract

Objective—Impaired basal nitric oxide release is associated with a number of cardiovascular disorders including hypertension, arterial spasm, and myocardial infarction. We determined whether basal endothelial nitric oxide release is reduced in otherwise healthy overweight and obese adult humans.

Research Methods and Procedures—Seventy sedentary adults were studied: 32 **normal weight** (< 25 kg/m^2), 24 overweight (BMI $\geq 25 < 30$ kg/m^2) and 14 obese (BMI: ≥ 30 kg/m^2). Forearm blood flow (FBF) responses to intra-arterial infusions of N^G-monomethyl-$_L$-arginine ($_L$-NMMA; 5 mg/min), a nitric oxide synthase inhibitor, were used as an index of basal nitric oxide release.

Results— $_L$-NMMA elicited significant reductions in FBF in the lean (from 4.1±0.2 to 2.8±0.2 mL/100 mL tissue/min), overweight (4.1±0.2 to 2.8±0.2 mL/100 mL tissue/min), and obese (3.9±0.3 to 2.7±0.2 mL/100 mL tissue/min) subjects. Importantly, the magnitude of reduction in FBF (~30%) was similar among the groups.

Discussion—These results indicate that the capacity of the endothelium to release nitric oxide under basal conditions is not compromised in overweight and obese adults.

Keywords

Overweight; Obesity; Nitric Oxide

Introduction

Endothelium-derived nitric oxide is a critical regulator molecule involved in the control of vasomotor tone both at rest and in response to various vasodilator stimuli (both physiologic and pharmacologic) (1). Moreover, nitric oxide is an important anti-atherogenic agent inhibiting vascular smooth muscle proliferation, platelet activation and aggregation, and leukocyte chemotaxis and adhesion to the endothelial surface (2). Reduced basal and stimulated endothelial nitric oxide bioavailability is thought to contribute etiologically to cardiovascular disease and its clinical consequences including hypertension, coronary vasospasm, and myocardial infarction in humans (3).

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Several studies (4,5) have shown that endothelium-dependent vasodilation evoked by the stimulated release of nitric oxide (via intra-arterial infusion of acetylcholine or methacholine) is impaired in overweight and obese adults. Reduced stimulated endothelial nitric oxide release, however, does not necessarily reflect a concomitant impairment in basal production. For example, in hypercholesterolemic patients stimulated nitric oxide-mediated vasodilation has been shown to be diminished, whereas, basal release is preserved (6). These and other data (7) suggest that basal and stimulated nitric oxide release may be regulated by different mechanisms and/or independently affected by different physiological states. The aim of the present investigation was to determine if basal endothelial nitric oxide release is reduced in overweight and obese adult humans. If so, this may contribute to the obesity-related increase in cardiovascular risk.

Research Methods and Procedures

Subjects

Seventy sedentary adults (46 men; 24 women) aged 44–71 years were studied: 32 normal weight (< 25 kg/m^2), 24 overweight (BMI ≥ 25 < 30 kg/m^2) and 14 obese (BMI: ≥ 30 kg/m^2). 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 (DPXIQ Lunar Radiation Corporation, Madison, WI). Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Minimal waist circumference was measured according to previously published guidelines (8). All subjects were free of overt cardiovascular and metabolic disease as assessed by medical history, physical examination, resting and exercise electrocardiograms and fasting blood chemistries. Insulin resistance was estimated using the homeostasis model assessment (HOMA IR) (9). None of the subjects smoked or were taking medication including vitamins. All of the women were postmenopausal and not taking hormone replacement therapy. Plasma concentrations of oxidized low-density lipoprotein (oxLDL), C-reactive protein (CRP) and tumor necrosis factor (TNF)-α were determined by enzyme immunoassay. The subjects had the research study and potential risks and benefits explained fully before providing written informed consent according to the guidelines of the University of Colorado at Boulder.

Experimental Design

All studies were performed between 7:00 am and 10:00 am after a 12- hour overnight fast. Under strict aseptic conditions a 5-cm 20-gauge catheter was inserted into the brachial artery of the nondominant arm under local anesthesia (2% lidocaine). Forearm blood flow (FBF) was measured in both the experimental (nondominant) and contralateral (dominant) forearm, via strain-gauge venous occlusion plethysmography. To assess basal nitric oxide release, N^G-monomethyl-L-arginine (L-NMMA; Clinalfa AG) was infused at 5 mg/min for 5 min (10). FBF was recorded during the last minute of L-NMMA infusion and the mean value reported. Prior to the beginning of this study, permission was obtained from the Food and Drug Administration to administer L-NMMA to humans.

Statistical Analysis

Subject baseline characteristics were analyzed by between-groups analysis of variance (ANOVA). Changes in FBF in response to L-NMMA 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 among the groups. There were no significant sex differences in any of the key outcome variables, therefore the data were pooled and presented together. All data are expressed as mean±SEM. Statistical significance was set at $P<0.05$.

Results

Selected subject characteristics are presented in the table. All indices of body composition were highest in the obese subjects. Although well within clinically normal ranges the overweight and obese subjects demonstrated higher ($P<0.05$) resting diastolic blood pressure, plasma triglyceride and insulin concentrations as well as HOMA IR levels and lower HDL-C concentrations compared with the normal weight controls. In addition, plasma concentrations of oxLDL, CRP and TNF-α, were higher in the overweight and obese subjects. Nine overweight and obese subjects met clinical criteria for the metabolic syndrome according to NCEP ATP III criteria (11).

There were no differences in resting FBF among the groups. L-NMMA produced significant reductions in FBF in all groups. Importantly, the magnitude of change was not significantly different among the groups ($P=0.36$)(Figure). FBF decreased ~30% in response to L-NMMA in the normal weight (from 4.1±0.2 to 2.7±0.2 mL/100 mL tissue/min), overweight (4.1±0.1 to 2.8±0.2 mL/100 mL tissue/min), and obese (3.9±0.3 to 2.7±0.2 mL/100 mL tissue/min) subjects. The response to LNMMA was not different in those subjects with the metabolic syndrome. There were no significant changes in FBF throughout the infusion protocol in the contralateral arm of any group. In the overall study population no significant univariate correlations were observed between the peak change (absolute and relative) in FBF to L-NMMA and any anthropometric, metabolic, or inflammatory variable.

Discussion

Endothelial cells produce nitric oxide from the guanidine-nitrogen terminal of the nonessential amino acid L-arginine by the enzyme nitric oxide synthase (12). In the present study the forearm vascular responses to a non-systemic dose of L-NMMA, a potent competitive stereospecific inhibitor of endothelial nitric oxide synthase, was used as an index of basal endothelial nitric oxide release (13). Our results indicate that basal release of nitric oxide is not impaired in either overweight or obese adults. Indeed, both groups demonstrated almost identical reductions in FBF to L-NMMA compared with their normal weight counterparts.

The results of the present study are in stark contrast to the well-reported reduction in stimulated endothelial nitric oxide release in overweight and obese adults. For example, Higashi and colleagues (4) have shown that the vasodilator response to the endothelial agonist acetycholine is largely unaffected by L-NMMA in overweight and obese adults, indicating diminished acetylcholine-stimulated nitric oxide release. The mechanisms responsible for reduced stimulated release of nitric oxide with overweight/obesity are not clear. In general reduced nitric oxide bioavailability has been attributed to either impaired production or increased degradation (14). The fact that we observed no group differences in vascular responses to endothelial nitric oxide synthase inhibition with L-NMMA argues against a primary deficiency in the L-arginine pathway to produce nitric oxide under basal conditions with obesity. However, when this pathway is stressed in response to a stimulus (physiologic or pharmacologic) defects may emerge that restrict the capacity of the endothelium to release nitric oxide.

A salient finding of the present study is that despite higher plasma concentrations of oxLDL, CRP and TNF-α, specific markers of oxidative stress and inflammation, basal nitric release was not compromised in our overweight and obese subjects. It has been suggested that obesity-related increases in inflammatory and oxidative stress exert deleterious effects on both nitric oxide synthesis and bioactivity (14). Our findings do not completely support this postulate. We are aware of no other data relating biomarkers of oxidative and inflammatory stress to basal nitric oxide release in overweight and obese adult humans.

In summary, we have demonstrated that basal endothelial nitric oxide release is not impaired in overweight and obese adults. Additional studies are needed to identify the defect in the nitric oxide pathway responsible for diminished stimulated release in this at risk population.

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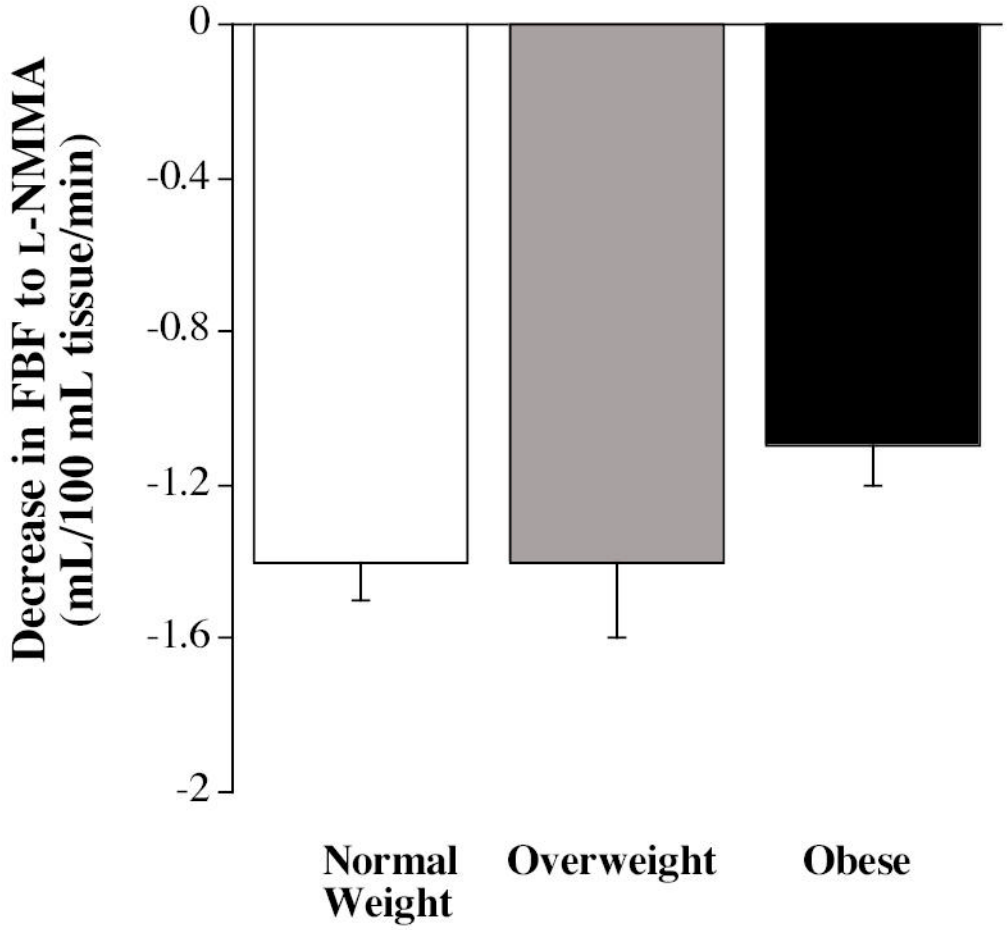

Figure.
Decrease in FBF from baseline in response to L-NMMA in lean, overweight and obese adults (P=0.36 across the groups). Values are mean±SEM.

Table 1

Selected subject characteristics.

Variable	Normal Weight (n=32)	Overweight (n=24)	Obese (n=14)
Age, yr	57±1	58±2	55±2
Gender	18 M/14 F	18 M/6 F	10 M/4 F
Body mass, kg	69.3±2.0	84.5±1.7*	100.6±3.8*†
BMI, kg/m^2	22.9±0.3	28.0±0.1*	33.1±1.0*†
Body fat, %	25.9±1.3	33.4±1.7*	38.3±2.0*†
Waist Circumference, cm	82.3±1.6	95.5±1.4*	109.3±2.8*†
Systolic BP, mmHg	114±2	120±2	120±2
Diastolic BP, mmHg	71±2	76±1*	80±2*
Total cholesterol, mmol/L	4.8±0.1	5.1±0.2	5.3±0.3
HDL-Cholesterol, mmol/L	1.5±0.1	1.2±0.1*	1.0±0.1*
LDL-Cholesterol, mmol/L	3.0±0.1	3.3±0.2	3.5±0.2
Triglycerides, mmol/L	1.0±0.1	1.5±0.1*	1.8±0.1*
Glucose, mmol/L	5.0±0.1	5.2±0.1	5.2±0.1
Insulin, pmol/L	26.4±1.5	32.0±2.9*	51.4±3.8*
HOMA IR	1.0±0.1	1.3±0.1*	2.0±0.2*†
oxLDL, U/L	42.6±2.2	60.3±4.7*	65.7±5.0*
C-Reactive Protein, ng/mL	1.0±0.2	1.7±0.3*	3.7±0.9*†
TNF-α, pg/mL	1.0±0.1	2.2±0.3*	1.8±0.3*

Mean±SEM

*P<0.05 vs Normal Weight

†P<0.05 vs Overweight