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Impaired endothelium-dependent vasodilation in overweight and obese adult humans is not limited to muscarinic receptor agonists

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Abstract

Muscarinic receptor agonists have primarily been used to characterize endothelium-dependent vasodilator dysfunction with overweight/obesity. Reliance on a single class of agonist, however, yields limited, and potentially misleading, information regarding endothelial vasodilator capacity. The aims of this study were to determine 1) whether the overweight/obesity-related reduction in endothelium-dependent vasodilation extends beyond muscarinic receptor agonists and 2) whether the contribution of nitric oxide (NO) to endothelium-dependent vasodilation is reduced in overweight/obese adults. Eighty-six middle-aged and older adults were studied: 42 normal-weight (54 ± 1 yr, 21 men and 21 women, body mass index = 23.4 ± 0.3 kg/m²) and 44 overweight/obese (54 ± 1 yr, 28 men and 16 women, body mass index = 30.3 ± 0.6 kg/m²) subjects. Forearm blood flow (FBF) responses to intra-arterial infusions of acetylcholine in the absence and presence of the endothelial NO synthase inhibitor *N*^G-monomethyl-L-arginine, methacholine, bradykinin, substance P, isoproterenol, and sodium nitroprusside were measured by strain-gauge plethysmography. FBF responses to each endothelial agonist were significantly blunted in the overweight/obese adults. Total FBF (area under the curve) to acetylcholine (50 ± 5 vs. 79 ± 4 ml/100 ml tissue), methacholine (55 ± 4 vs. 86 ± 5 ml/100 ml tissue), bradykinin (62 ± 5 vs. 85 ± 4 ml/100 ml tissue), substance P (37 ± 4 vs. 57 ± 5 ml/100 ml tissue), and isoproterenol (62 ± 4 vs. 82 ± 6 ml/100 ml tissue) were 30%–40% lower in the overweight/obese than normal-weight adults. *N*^G-monomethyl-L-arginine significantly reduced the FBF response to acetylcholine to the same extent in both groups. There were no differences between the groups in the FBF responses to sodium nitroprusside. These results indicate that agonist-stimulated endothelium-dependent vasodilation is universally impaired with overweight/ obesity. Moreover, this impairment appears to be independent of NO.

Keywords

cell surface receptors; intracellular signaling pathways; nitric oxide

Impaired endothelium-dependent vasodilation, a hallmark characteristic of endothelial dysfunction, has been linked etiologically to the initiation and development of atherosclerotic vascular disease (25, 35). Indeed, reduced endothelium-mediated vasodilation occurs early in atherogenesis, before histological and/or angiographic evidence of disease (38). Endothelial function, particularly endothelium-dependent vasodilation, is impaired in overweight and obese adults and is thought to contribute to their increased risk of coronary artery disease, cerebrovascular disease, and atherothrombotic events (23, 24, 29, 30, 32)

The mechanisms responsible for the adiposity-related reduction in endothelial vasodilator function in adult humans are not completely understood. Pharmacological studies have primarily employed muscarinic receptor agonists, acetylcholine or methacholine, to assess the influence of increased body fatness on endothelium-dependent vasodilation (23, 30, 31). The reliance on a single agonist, however, yields limited, and potentially misleading, information regarding endothelial vasodilator capacity. For example, we previously demonstrated that although the forearm vasodilator response to acetylcholine declines with advancing age, the vascular responses to other agonists, including bradykinin, substance P, and isoproterenol, are well preserved (9). Moreover, in hypercholesterolemic patients, forearm vasodilation to acetylcholine and substance P, but not bradykinin, has been shown to be blunted (6, 14). Thus the use of multiple agonists is a more rigorous approach to assess endothelial vasodilator capacity in conscious humans, inasmuch as it provides additional insight into the extent, degree, and potential mechanism of dysfunction. It is unknown whether the endothelial vasodilator dysfunction observed in overweight and obese adults is limited to muscarinic receptor agonists.

Accordingly, the primary experimental aim of the present study was to determine whether the overweight/obesity-related reduction in endothelium-dependent vasodilation extends beyond muscarinic receptor agonists. To address this aim, we determined the forearm vascular responses to a variety of endothelial agonists that stimulate nitric oxide (NO)-mediated vasodilation via different cell surface receptors and intracellular signaling pathways in normal-weight and overweight/obese adults. A secondary aim was to determine whether the contribution of NO to endothelium-dependent vasodilation is reduced in overweight/obese adults.

METHODS

Subjects

Eighty-six middle-aged and older adults (41–71 yr of age) were studied: 42 were normal weight [body mass index (BMI) ≤ 25.0 kg/m²] and 44 were overweight/obese (BMI = 27.0–40.0 kg/m²). All subjects were sedentary and had not participated in a regular aerobic exercise program for ≥ 2 yr before the start of the study. Subjects were excluded from the study if they presented a history or evidence of hepatic, renal, or hematologic disease; peripheral vascular disease; stroke; diabetes (fasting plasma glucose >7.0 mmol/l) (2); dyslipoproteinemia (total cholesterol ≥ 6.0 mmol/l, triglycerides ≥ 3.0 mmol/l) (11); or hypertension (blood pressure $\geq 140/90$ mmHg) (7). All subjects were screened for clinical evidence of coronary artery disease with resting and maximal-exercise electrocardiograms and blood pressure. None of the subjects smoked or were taking medication, including vitamins. All the women were ≥ 1 yr (mean 7 ± 2 yr) postmenopause and had never used hormone replacement therapy or discontinued use of hormone replacement therapy ≥ 1 yr before the start of the study. The research study and its potential risks and benefits were explained fully before the subjects provided written informed consent according to the guidelines of the University of Colorado at Boulder. This study was approved by the University of Colorado at Boulder Human Research Committee.

Measurements

Body composition—Body mass was measured to the nearest 0.1 kg with 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 (16).

Treadmill exercise test—For assessment of aerobic fitness, subjects performed incremental treadmill exercise using a modified Balke protocol as previously described (10). Maximal oxygen consumption ($\dot{V}O_{2\max}$) 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 derived from fasting glucose and insulin concentrations (18). Plasma concentrations of oxidized low-density lipoprotein (oxLDL), C-reactive protein (CRP), and asymmetric dimethylarginine (ADMA) were determined by enzyme immunoassay (33).

Intra-arterial infusion protocols—All measurements were performed in a temperature-controlled room between 7 and 10 AM after a 12-h 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 (1% lidocaine). Forearm blood flow (FBF) was measured in the experimental (nondominant) and the contralateral (dominant) forearm using strain-gauge venous occlusion plethysmography (D. E. Hokanson) as previously described (9). FBF responses are presented in milliliters per 100 milliliters of forearm volume per minute. The total amount of blood flow across the forearm in response to each agonist was calculated as the area under each curve above baseline with use of a trapezoidal model. Before the beginning of the study, permission was obtained from the US Food and Drug Administration to administer methacholine, bradykinin, substance P, and *N*^G-monomethyl-L-arginine (L-NMMA) to humans.

Protocol 1—In 23 of the 42 normal-weight ($BMI = 23.4 \pm 0.4 \text{ kg/m}^2$, 11 men and 12 women) and 24 of the 44 overweight/obese ($BMI = 30.3 \pm 0.7 \text{ kg/m}^2$, 15 men and 9 women) subjects, endothelium-dependent vasodilation was assessed by FBF responses to incremental doses of acetylcholine (IOLAB Pharmaceuticals, Duluth, GA), methacholine (Clinalfa), bradykinin (Clinalfa), substance P (Clinalfa), and isoproterenol (Isuprel, Abbott Laboratories). FBF response to sodium nitroprusside (Nitropress, Abbott Laboratories) was used to assess endothelium-independent vasodilation. Acetylcholine was infused at 4.0, 8.0, and 16.0 $\mu\text{g} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$, methacholine at 0.15, 0.30 and 1.0 $\mu\text{g} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$, bradykinin at 12.5, 25.0, and 50.0 $\text{ng} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$, substance P at 75, 150, and 300 $\text{pg} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$, isoproterenol at 5, 10, and 20 $\text{ng} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$, and sodium nitroprusside at 1.0, 2.0, and 4.0 $\mu\text{g} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$. These doses of acetylcholine, methacholine, bradykinin, substance P, and isoproterenol have been shown to elicit comparable increases in FBF in healthy adults (5, 9, 20). Each dose was infused for ~5 min, and sufficient time (~20 min) was allowed for FBF to return to resting levels between drug infusions. To avoid an order effect, the sequence of drug administration was randomized.

Protocol 2—FBF responses to acetylcholine were determined before and after administration of the endothelial NO synthase (eNOS) inhibitor L-NMMA (Clinalfa). These

studies were performed in 19 normal-weight ($\text{BMI} = 23.3 \pm 0.4 \text{ kg/m}^2$, 10 men and 9 women) and 20 overweight/obese ($\text{BMI} = 30.2 \pm 0.9 \text{ kg/m}^2$, 13 men and 7 women) subjects who did not participate in *protocol 1*. After acetylcholine was infused at the doses noted above and blood flow was allowed to return to resting levels, L-NMMA was infused at $2.5 \text{ mg} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$ for 5 min. Immediately thereafter, the acetylcholine dose response was repeated with the continuous infusion of L-NMMA.

Statistical Analysis

Differences in subject baseline characteristics and area under the curve data were determined by ANOVA. Group differences in FBF responses to each vasoactive drug were determined by repeated-measures ANOVA. Because there were no significant sex differences with respect to the main effect of overweight/obesity on any of the key outcome variables, the data were pooled and are presented together. Also, because there were no significant differences in the responses to the vasoactive agents between the overweight ($\text{BMI} = 27\text{--}29.9 \text{ kg/m}^2$) and obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) adults, the groups were combined and referred to as overweight/obese. The data were analyzed using JMP 6 statistical software (SAS, Cary, NC). Values are means $\pm$ SE. Statistical significance was set a priori at $P < 0.05$.

RESULTS

Table 1 presents selected subject characteristics. By design, body mass, BMI, and waist circumference were higher ($P < 0.01$) in the overweight/obese subjects than normal-weight controls. In addition, although well within clinically normal levels, plasma triglyceride, glucose, and insulin concentrations were higher in the obese subjects. Circulating concentrations of ADMA were not different, but CRP and oxLDL levels were higher in the overweight/obese subjects than normal-weight controls.

Protocol 1: FBF Responses to Acetylcholine, Methacholine, Bradykinin, Substance P, Isoproterenol, and Sodium Nitroprusside

Resting FBF in the noninfused arm and mean arterial pressure remained constant throughout the infusion protocols and were not significantly different between the groups. Blood flow in the infused arm returned to baseline levels after the infusion of each agonist and was not significantly different between the groups.

Acetylcholine—Figure 1 shows the FBF responses to acetylcholine in the normal-weight and overweight/obese groups. The overweight/obese subjects demonstrated a markedly blunted vasodilator response to acetylcholine. The increase in the FBF response to acetylcholine was $\sim 25\%$ less ($P < 0.01$) in the overweight/obese subjects (from 4.3 ± 0.2 to $11.4 \text{ ml} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$) than normal-weight controls (from 4.5 ± 0.2 to $15.4 \text{ ml} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$). As a result, total FBF to acetylcholine (area under the FBF curve) was $\sim 40\%$ lower in the overweight/obese subjects than normal-weight controls (50 ± 5 vs. $79 \pm 4 \text{ ml}/100 \text{ ml tissue}$).

Methacholine—Methacholine was administered to 22 of the 23 normal-weight controls and 21 of the 24 overweight/obese subjects because of drug availability. Similar to acetylcholine, FBF responses to methacholine were significantly related to differences in adiposity (Fig. 2). The FBF response to methacholine was $\sim 25\%$ lower ($P < 0.01$) in the overweight/obese subjects (from 4.2 ± 0.2 to $12.7 \pm 0.6 \text{ ml} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$) than normal-weight controls (from 4.6 ± 0.2 to $17.3 \pm 0.6 \text{ ml} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$). In addition, total FBF to methacholine was $\sim 30\%$ lower ($P < 0.05$) in the overweight/obese subjects than normal-weight controls (55 ± 4 vs. $86 \pm 5 \text{ ml}/100 \text{ ml tissue}$).

Bradykinin—FBF responses to bradykinin are shown in Fig. 3. In response to bradykinin stimulation, FBF was ~20% lower ($P < 0.01$) in the overweight/obese subjects (from 4.1 ± 0.3 to 12.9 ± 0.7 ml · 100 ml tissue⁻¹ · min⁻¹) than normal-weight controls (from 4.6 ± 0.2 to 16.4 ± 0.6 ml · 100 ml tissue⁻¹ · min⁻¹). Consequently, total FBF to bradykinin was lower (~25%, $P < 0.05$) in the overweight/obese subjects than normal-weight controls (62 ± 5 vs. 85 ± 4 ml/100 ml tissue).

Substance P—Figure 4 shows the FBF responses to substance P in the normal-weight and overweight/obese groups. The obese subjects demonstrated significantly reduced vasodilator responses to substance P: the FBF response to substance P was ~25% lower in the overweight/obese subjects (from 4.2 ± 0.2 to 10.6 ± 0.6 ml · 100 ml tissue⁻¹ · min⁻¹) than normal-weight controls (from 4.6 ± 0.2 to 13.4 ± 0.8 ml · 100 ml tissue⁻¹ · min⁻¹). Total FBF to substance P was ~35% less in the overweight/obese subjects than normal-weight controls (37 ± 4 vs. 57 ± 5 ml/100 ml tissue).

Isoproterenol—Similar to acetylcholine, methacholine, bradykinin, and substance P, forearm vascular responses to isoproterenol were diminished in the overweight/obese subjects (Fig. 5). The increase in the FBF response to isoproterenol was ~25% lower ($P < 0.01$) in the obese subjects (from 4.4 ± 0.2 to 13.8 ± 0.8 ml · 100 ml tissue⁻¹ · min⁻¹) than normal-weight controls (from 4.7 ± 0.2 to 16.9 ± 0.8 ml · 100 ml tissue⁻¹ · min⁻¹). Total FBF to isoproterenol was significantly (~25%) less in the overweight/obese subjects than normal-weight controls (62 ± 4 vs. 82 ± 6 ml/100 ml tissue).

Sodium nitroprusside—There were no significant differences between the groups in the FBF responses to sodium nitroprusside (Fig. 6).

Protocol 2: Effects of L-NMMA on FBF Responses to Acetylcholine

Consistent with our findings in *protocol 1*, FBF responses to acetylcholine were significantly lower in the overweight/obese subjects (from 4.3 ± 0.2 to 11.1 ± 0.7 ml · 100 ml tissue⁻¹ · min⁻¹) than normal-weight controls (from 4.6 ± 0.3 to 14.2 ± 0.9 ml · 100 ml tissue⁻¹ · min⁻¹). L-NMMA produced similar reductions (~30%) in FBF at baseline in the overweight/obese subjects (from 4.2 ± 0.2 to 2.6 ± 0.2 ml · 100 ml tissue⁻¹ · min⁻¹) and normal-weight controls (from 4.3 ± 0.2 to 2.7 ± 0.2 ml · 100 ml tissue⁻¹ · min⁻¹). Infusion of L-NMMA significantly blunted the acetylcholine-mediated vasodilator response in both groups (Fig. 7). The magnitude of the reduction (~20%) in FBF was not significantly different between the overweight/obese subjects and normal-weight controls.

DISCUSSION

It is well established that the forearm vasodilator response to acetylcholine and methacholine is significantly impaired in overweight and obese adult humans (23, 30, 31). The results of the present study confirm and extend these findings, inasmuch as we demonstrate for the first time that FBF responses to other endothelial agonists are also significantly impaired with increased adiposity. Indeed, in addition to acetylcholine and methacholine, forearm vasodilation to bradykinin, substance P, and isoproterenol was substantially lower (~30%) in overweight/obese adults than their normal-weight peers. Moreover, the results of the present study suggest that impaired endothelium-dependent vasodilation with increased adiposity is not due to diminished NO production.

To comprehensively assess the influence of overweight/ obesity on endothelium-dependent vasodilation in conscious humans, we determined the FBF responses to a variety of endothelial agonists that act via different endothelial cell surface receptors, intracellular

membrane-bound G proteins, and signal transduction pathways (27). Acetylcholine and methacholine are muscarinic receptor agonists that stimulate the phospholipase C-phosphatidylinositol- Ca^{2+} signaling pathway, via the intracellular membrane-bound pertussis toxin-sensitive G protein, leading to increased intracellular Ca^{2+} and the activation of eNOS and, in turn, production and subsequent release of NO (4, 27). Similar to acetylcholine and methacholine, bradykinin stimulates the phospholipase C-phosphatidylinositol- Ca^{2+} intracellular signal transduction pathway; however, this is mediated through B2-kininergic receptors coupled to a pertussis toxin-insensitive G protein (3). Substance P and acetylcholine share the same G protein signaling pathway, but substance P interacts with tachykinin receptors on the endothelial cell surface (26). Inasmuch as acetylcholine, methacholine, substance P, and bradykinin share the same phospholipase C-phosphatidylinositol- Ca^{2+} intracellular signal transduction pathway, it is possible that impaired Ca^{2+} release may contribute to the impaired responses. However, isoproterenol is a β -adrenoceptor agonist that stimulates vasorelaxation, in part, by activating the adenylyl cyclase pathway, resulting in cAMP activation of eNOS (5). Thus, in the present study, regardless of the agonist administered, endothelium-dependent vasodilation was significantly reduced in the overweight/obese subjects compared with normal-weight controls. Moreover, the magnitude of impairment in vasodilation was similar among the agonists, suggesting a general endothelial vasomotor abnormality, rather than a specific endothelial receptor or signal transduction pathway defect. Consistent with this postulate, we recently reported that obesity-related impairment in acetylcholine-mediated vasodilation with overweight/obesity is not due to reduced muscarinic receptor responsiveness or sensitivity (32).

The mechanisms responsible for this general dysfunction in agonist-mediated endothelium-dependent vasodilation in overweight/obese adults are not clear. All the endothelial agonists used in the present study stimulate vasodilation, at least in part, by the activation of eNOS and subsequent production of NO. Overweight and obesity are conditions characterized by increased oxidative stress and inflammatory burden (12, 15). Increased oxidative stress and systemic inflammation have been shown to have unfavorable effects on stimulated endothelial NO bioavailability (37). Consistent with previous work (12, 15), our overweight/obese subjects demonstrated higher circulating plasma concentrations of oxLDL and CRP, specific markers of oxidative and inflammatory stress, respectively. Thus a reduction in NO bioavailability would be a logical mechanism underlying the global impairment in vasodilator capacity in the overweight/obese subjects. However, the results of the present study do not support this postulate. In response to infusion of acetylcholine + L-NMMA, we observed almost identical reductions in forearm vasodilation in the normal-weight controls and overweight/obese subjects, suggesting that the contribution of NO to acetylcholine-mediated vasodilation is preserved with increased adiposity. In addition, we observed no differences in circulating levels of the endogenous eNOS inhibitor ADMA or resting FBF responses to L-NMMA between the overweight/obese subjects and normal-weight controls. Consistent with our findings, Nielsen et al. (22) reported similar FBF responses to L-NMMA in a comparison of young normal-weight and upper body-obese men. Complementing these findings, a recent in vitro study involving endothelial cells isolated from normal-weight and overweight/obese adults revealed no group differences in eNOS protein expression (28). Thus, taken together, these data suggest that adiposity-related endothelial vasodilator dysfunction is not due to impaired eNOS activity or NO production.

It is possible that elevated vasoconstrictor tone may blunt endothelial vasodilator capacity in overweight/obese adults. For example, endothelin-1 (ET-1)-mediated vasoconstriction has been reported to be elevated in overweight/obese adults. ET-1, which is produced primarily by vascular endothelial cells, is one of the most potent endogenous vasoconstrictor peptides (36). Mather et al. (17) showed that ET-1-mediated vasoconstrictor tone is higher in

overweight/obese than normal-weight adults and that this increase in vasoconstrictor activity contributes to the endothelial vasodilator dysfunction associated with increased body fatness. Specifically, they demonstrated that blockade of the ET_A receptor restored leg blood flow responses to methacholine in overweight and obese adults to levels similar to that of normal-weight controls. In addition, angiotensin II-stimulated forearm vasoconstriction has been reported to be higher in obese men (22). Thus an increase in endothelial vasoconstrictor tone resulting in limited vasodilator capacity is a tenable hypothesis to account for the general impairment in endothelium-dependent vasodilation in the overweight/obese adults.

From a clinical perspective, it is important to emphasize that there were no significant differences in the FBF responses to any of the endothelial agonists (or sodium nitroprusside) between the overweight (mean BMI = 27.7 ± 0.3 kg/m²) and obese (BMI = 33.4 ± 0.8 kg/m²) subjects within the overweight/obese group. Indeed, the peak FBF response to acetylcholine (12.3 ± 0.7 vs. 10.4 ± 1.0 $\mu\text{g} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$), methacholine (12.3 ± 0.8 vs. 12.9 ± 0.8 $\mu\text{g} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$), bradykinin (13.2 ± 0.5 vs. 11.7 ± 1.0 $\mu\text{g} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$), substance P (11.3 ± 0.9 vs. 9.9 ± 0.8 ml/100 ml tissue), and isoproterenol (14.3 ± 1.0 vs. 13.1 ± 1.0 ml/100 ml tissue) was similar between the groups, irrespective of the marked difference in BMI. These data indicate that the impaired endothelial vasodilator function commonly observed with obesity is apparent in the overweight state. This finding complements a previous study from our laboratory (34), in a comparable study population, demonstrating similar levels of endothelial fibrinolytic dysfunction in overweight and obese subjects compared with normal-weight adults. Thus increased adiposity resulting in an overweight or obese body status classification is associated with unfavorable changes in vascular endothelial function. Inasmuch as ~65% of the US population is overweight (13) and endothelial vasodilator and fibrinolytic dysfunction are key antecedents to cardiovascular disease and acute vascular events (19, 21, 25, 29), it is imperative that interventions aimed at reducing adiposity and improving endothelial function not only target obese adults but also those who are mild to moderately overweight.

Three experimental considerations regarding this study should be mentioned. 1) Considering our cross-sectional study design, we cannot dismiss the possibility that genetic and/or lifestyle behaviors may have influenced the results of our group comparisons. To minimize the influence of lifestyle behaviors, all subjects were nonsmokers, were not currently taking medication that could influence endothelium-dependent vasodilation, and did not differ in habitual physical activity. In addition, we studied carefully screened normal-weight and overweight/obese adults to eliminate the confounding effects of clinically overt cardiovascular and metabolic disease. 2) Although we determined the influence of overweight/obesity on forearm endothelium-dependent vasodilation responses to a number of agonists, the role of NO in the vasodilator responses was only assessed using L-NMMA with acetylcholine. Inasmuch as the contribution of NO to endothelium-mediated vasodilation can vary substantially depending on the agonist used, we cannot confidently infer from our results similar findings among the other agents. For example, up to 70% of the forearm vasodilation induced by substance P has been shown to be NO mediated, compared with 30–40% for acetylcholine (5, 8, 20). It is possible that overweight/obesity-related differences in NO-mediated vasodilation may emerge with agonists that are predominantly NO dependent, such as substance P. 3) The results of the present study pertain only to the forearm vasculature. Although acetylcholine- and methacholine-mediated endothelium-dependent vasodilation has been shown to be impaired in the coronary and femoral arteries of overweight/obese adults (1, 31), it is unknown whether the extent of dysfunction observed in the forearm in response to various agonists is similar to that in other vascular beds.

In conclusion, the results of the present study demonstrate that increased adiposity is associated with profound impairment in endothelium-dependent vasodilation that extends beyond muscarinic receptor agonists. Regardless of the endothelial agonist employed, forearm vasodilator capacity is markedly lower in overweight/obese than normal-weight adults. Moreover, the impairment in endothelial vasodilation with overweight/obesity appears to be independent of NO bioavailability. Clinically, it is important to emphasize that the degree of endothelial vasodilator dysfunction observed with obesity is evident in the overweight state, suggesting that any excess adiposity is associated with impaired endothelial vasodilator function.

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Fig. 1. Forearm blood flow (FBF; *A*) responses and total FBF (area under the curve; *B*) to acetylcholine in normal-weight and overweight/obese groups. Values are means $\pm$ SE. * $P < 0.05$ vs. normal weight.

Fig. 2. FBF responses (*A*) and total FBF (area under the curve; *B*) to methacholine in normal-weight and overweight/obese groups. Values are means $\pm$ SE. * $P < 0.05$ vs. normal weight.

Fig. 3. FBF responses (*A*) and total FBF (area under the curve; *B*) to bradykinin in normal-weight and overweight/obese groups. Values are means $\pm$ SE. * $P < 0.05$ vs. normal weight.

Fig. 4. FBF responses (*A*) and total FBF (area under the curve; *B*) to substance P in normal-weight and overweight/obese groups. Values are means $\pm$ SE. * $P < 0.05$ vs. normal weight.

Fig. 5. FBF responses (*A*) and total FBF (area under the curve; *B*) to isoproterenol in normal-weight and overweight/obese groups. Values are means $\pm$ SE. * $P < 0.05$ vs. normal weight.

Fig. 6. FBF responses (*A*) and total FBF (area under the curve; *B*) to sodium nitroprusside in normal-weight and overweight/obese groups. Values are means $\pm$ SE.

Fig. 7. FBF responses and total FBF (area under the curve) to acetylcholine in the absence and presence of the nitric oxide (NO) synthase inhibitor *N*^G-monomethyl-L-arginine (L-NMMA) in normal-weight and overweight/obese groups. Values are means ± SE **P* < 0.05 vs. saline.

Table 1

Selected subject characteristics

Variable	Normal Weight (n = 42)	Overweight/Obese (n = 44)
Age, yr	54±1	54±1
Sex, M/F	21/21	28/16
Body mass, kg	69.9±1.7	90.8±2.2 *
Body fat, %	28.3±1.0	36.0±1.2 *
BMI, kg/m ²	23.4±0.3	30.3 ±0.6 *
Waist circumference, cm	81.8±1.4	100.7±2.1 *
BP, mmHg		
Systolic	117±2	122±1 *
Diastolic	76±1	78±1
Max O ₂ uptake, l/min	2.3±0.1	2.5±0.1
Total cholesterol, mmol/l	5.1 ±0.6	5.2±1.1
LDL-C, mmol/l	3.2±0.6	3.3±0.9
HDL-C, mmol/l	1.4±0.3	1.2±0.3
TG, mmol/l	1.1±0.5	1.4±0.7 *
Glucose, mmol/l	4.9±0.5	5.2±0.5 *
Insulin, pmol/l	27.9±1.9	41.7±3.0 *
HOMA-IR	1.0±0.1	1.6±0.1 *
ADMA, µmol/l	0.56±0.03	0.50±0.04
CRP, mg/l	0.9±0.2	3.2±0.5 *
oxLDL, U/l	45.6±1.9	55.9±2.7 *

Values are means ± SE. BMI, body mass index; BP, blood pressure; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglycerides; HOMA-IR, homeostasis model assessment of insulin resistance; ADMA, asymmetric dimethylarginine; CRP, C-reactive protein; oxLDL, oxidized LDL.

* $P < 0.05$ vs. normal weight.