Adaptation of Vasomotor Function of Human Coronary Arterioles to the Simultaneous Presence of Obesity and Hypertension

Tibor Fulop, Eva Jebelovszki, Nora Erdei, Tamas Szerafin, Tamas Forster, Istvan Edes, Akos Koller, Zsolt Bagi

Objective—We hypothesized that simultaneous presence of obesity and hypertension activates adaptive vascular mechanisms affecting dilations of human coronary arterioles.

Methods and Results—Agonist-induced dilations were assessed in isolated pressurized coronary arterioles from patients (n=38) who underwent cardiac surgery. Among normotensives we found that dilations to bradykinin (BK) and the NO-donor, sodium-nitroprusside (SNP) were reduced in obese subjects (BK, 10⁻⁷ mol/L, lean:90±4%, obese:64±7%; SNP, 10⁻⁶ mol/L, lean:89±7%, obese:76±5%). However, among hypertensives, both BK- and SNP-induced dilations were significantly enhanced in obese patients, when compared with lean individuals (BK, lean:71±7%, obese:85±3%; SNP, lean:60±6%, obese:83±2%). Correspondingly, in hypertensive patients, but not in those of normotensives, a positive correlation was found between body mass index (BMI) and BK-induced (P=0.036, r=0.46), and also SNP-evoked (P=0.031, r=0.44) coronary dilations. Moreover, in additional 55 hypertensive patients flow-mediated (FMD) and nitroglycerin (NTG)-induced dilations of the brachial artery were assessed. In obese hypertensive individuals, FMD- and NTG-induced dilations were greater (FMD:6.2±0.7%, NTG:17.2±0.9%), than in lean hypertensive patients (FMD:3.7±0.6%, NTG:13.6±1.1%). Correspondingly, FMD- and NTG-induced dilations were positively correlated with BMI (P=0.020, r=0.31 and P=0.033, r=0.29, respectively).

Conclusions—These findings are the first to suggest that obesity may lead to activation of adaptive vascular mechanisms to enhance the dilator function of coronary and peripheral arterial vessels in hypertensive patients. (Arterioscler Thromb Vasc Biol. 2007;27:2348-2354.)

Key Words: obesity ■ hypertension ■ coronary microcirculation ■ flow-mediated dilation ■ nitrate

Comorbidity of obesity with other diseases, such as hypertension, is very common in the Western populations. Both obesity and hypertension are considered to be independent predictors of coronary heart disease (CHD). Previous studies demonstrated that vasomotor dysfunction of coronary vessels is one of the early alterations associated with CHD. In hypertension an early development of vasomotor dysfunction seems to be established by studies on experimental animals and humans vessels, which—in the early phase—is characterized primarily by reduced dilations of both coronary and peripheral arterial vessels. Recently, it has been shown that obese children already exhibit impaired brachial artery relaxation to hyperemic flow (flow-mediated dilation [FMD]) and a close association between coronary vasomotor function and FMD of brachial artery, it was speculated that obesity may also adversely affect coronary dilations.

There are few if any studies investigating the possible effects of hypertension and obesity on coronary microvascular vasomotor function in humans, but it seems logical to assume that simultaneous presence of hypertension and obesity adversely affects coronary arteriolar dilations, leading to disturbed regulation of blood flow hence predisposing these patients to tissue ischemia. Interestingly, early studies have postulated that any increase in body mass, such as adiposity, requires higher cardiac output, therefore at any level of systemic blood pressure vascular resistance is lower in obese than in lean patients. Recent studies on animal models of obesity are favoring this assumption, in which unexpectedly preserved or even enhanced vasodilations have been described in coronary resistance vessels. These findings led us to raise the hypothesis that hypertension and obesity may not simply have an additive deleterious effect. Alternatively, we also hypothesized that adaptive mechanisms intrinsic to vascular wall are activated aiming to maintain or enhance the dilator function of coronary arterioles during the development of diseases. In humans with hypertension and obesity the...
possible alterations in the vasomotor function of coronary arterioles have not yet been elucidated. Thus, the aim of this study was to investigate the impact of hypertension and obesity on dilations of human coronary arterioles to endothelium-dependent and -independent vasoactive agents with known mechanism of action. In addition, we aimed to test further the hypothesis that alterations in the vasomotor function of coronary microvessels are mirrored in that of large peripheral conduit arteries.

**Methods**

**Patients**

All protocols were approved by the Ethical Committee at the University of Debrecen, Medical and Health Science Center. All patients were given written information about experimental interventions and the experimental use of human specimen. Assessment of coronary arteriolar responses was performed in patients (n = 38, Table 1) who underwent cardiac surgery. Assessment of brachial artery relaxation was performed in a separate group of patients with documented hypertension (n = 55, Table 2), who were recruited locally.

**Isolation of Human Coronary Arterioles and Experimental Protocols**

With the use of microsurgical instruments and an operating microscope, the coronary arteriole (~1 mm in length) from the right atrial appendage were isolated and cannulated, as described previously. The cannulated arteriole was connected with silicone tubing to the pressure servo control system (Living Systems Instrumentation) to set the intraluminal pressure to 80 mm Hg. Changes in arteriolar diameter were continuously recorded with a digital camera (CPW1310, Scion Corp), connected to a microscope (Nikon, Eclipse 80i). During an incubation period of 1 hour, a spontaneous myogenic tone developed in the isolated coronary arterioles in response to the intraluminal pressure of 80 mm Hg. Then, the endothelium-dependent dilator bradykinin (0.1 nmol/L to 1 μmol/L, from Sigma) and the endothelium-independent dilator, nitric oxide donor, sodium nitroprusside (SNP; 1 nmol/L to 10 μmol/L, from Sigma) were administered to the arterioles, and the peak changes in diameter were measured. Concentrations of the drugs were selected on the basis of our previous study. Agonist-induced arteriolar responses were expressed as changes in arteriolar diameter as a percentage of the maximal dilation defined as the passive diameter of the vessel at 80 mm Hg intraluminal pressure in a Ca²⁺-free medium. At all drug concentrations data and correlation analyses were then performed and selected (representative) concentrations were presented.

**Assessment of Brachial Artery Relaxation to Hyperemic Flow (Flow Mediated Dilation) and to Nitroglycerin**

Ultrasound measurements of the brachial artery were performed according to the method described by Celermajer et al., using high-resolution ultrasound (Acuson Sequoia) with a 7.5-MHz linear array transducer. Diameter measurements of the right brachial artery were taken at rest after supine rest for at least 10 minutes, after cuff deflation completing suprasystolic compression (at least 50 mm Hg above systolic pressure) of the right upper arm for 4.5 minutes, and after sublingual application of 0.4 mg of nitroglycerin. Scans were taken of the brachial artery proximal to the bifurcation of the radial and the ulnar artery by the same ultrasound operator. Lumen diameters were measured from one media-adventitia interface to the other at least 3 times at baseline, every 20 seconds after reactive hyperemia, and subsequent to the administration of nitroglycerin. The maximum flow mediated dilation (FMD)- and nitroglycerin (NTG)-induced dilation diameters were taken as the average of the 3 consecutive maximum diameter measurements. Vasodilation was then calculated as the percent change in diameter over the baseline value.

**Statistical Analysis**

Data were stored and analyzed with the NCSS statistical software. Test selection was based after evaluating the variables for normal distribution, using the Kolmogorov-Smirnov test. Testing differ-
ences of different variables between normotensive and hypertensive groups was accomplished by 2-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. One-way ANOVA followed by Tukey posthoc test was performed to compare differences in brachial artery responses, which was investigated only in hypertensive patients. Because all the obtained data in this study met the normality criteria accomplished by Kolmogorov-Smirnov test, associations between continuous variables were analyzed by the Pearson correlation test. To examine categorical variables 2-way ANOVA was performed with the categorical variable and hypertension, as the 2 factors. Those associations which were significant on univariate analysis were entered into a multiple regression model, adjusted for the significant covariates. In a linear regression analysis the slopes of regression lines were also compared between normotensive and hypertensive groups. Data are expressed as means±SEM. P<0.05 was considered statistically significant.

Results

Dilations of Isolated Coronary Arterioles

Agonist-induced vasomotor responses were investigated in coronary arterioles isolated from patients (n=38) who underwent coronary bypass or valve replacement surgery. Patient demographics are summarized in Table 1. Patients were divided into normotensive (NT, n=14) and hypertensive (HT, n=24) subpopulation on the basis of the absence or presence of documented hypertension. Hypertensive patients exhibited higher actual blood pressure readings, which however did not reach statistical significance (Table 1). In the 2 groups there was no difference in the body mass index (BMI) and the prevalence of other underlying diseases, medications, as well as the age, gender, and the type of cardiac surgery (Table 1).

In isolated coronary arterioles a spontaneous tone developed in response to 80 mm Hg intraluminal pressure (active diameter: NT: 92±9 mm versus HT, 88±7 mm, P=0.256 and passive diameter in Ca²⁺ free solution: NT: 139±11 versus HT: 135±7, P=0.624). The endothelium-dependent vasodilator, bradykinin (0.1 nmol/L to 10 μmol/L) and the NO-donor, sodium nitroprusside (SNP, 1 nmol/L and 10 μmol/L) elicited substantial dilation in isolated coronary arterioles (Figure 1a and 1b). On the average, there were no significant differences between bradykinin-induced and SNP-evoked dilations of coronary arterioles isolated from normotensive and hypertensive individuals (Figure 1a and 1b). However, when the magnitudes of coronary dilations were segregated into lean (BMI<25) and obese (BMI≥30) patients groups a marked difference could be revealed by 2-way ANOVA between normotensive and hypertensive patients. Bradykinin-induced dilations were significantly reduced in obese-normotensive patients, when compared with lean-normotensive (P=0.013), and interestingly also when compared with those of obese-hypertensive patients (P=0.025, Figure 1c). It should be noted, that ANOVA also revealed a significant interaction between hypertension and obesity (P=0.001), thereby limiting the conclusions regarding to the independent effect of these variables on bradykinin-
induced responses. Moreover, we have found that the NO donor, SNP-induced dilations were significantly reduced in lean-hypertensive patients, when compared with lean-normotensive (non-HT, \(n=14\), empty symbols) and hypertensive (HT, \(n=24\), filled symbols) patients. Regression lines are included in the Figure that is dashed in non-HT and solid in HT groups.

To elucidate and further validate the possible role of overweight and obesity affecting coronary arteriolar responses the impact of BMI on the magnitude of dilations was also investigated by the Pearson correlation test. We have found a significant positive correlation between bradykinin-induced dilations and BMI in the hypertensive group, whereas a negative correlation was found in those of normo-tensive patients (Figure 2a). Similarly, a positive correlation was observed between SNP-induced dilations of coronary arterioles and BMI in hypertensive, but not in those of normotensive subjects (Figure 2b). The slopes of regression lines were also compared between normotensive and hypertensive groups. Evaluating bradykinin-induced responses, significant difference was found between the slopes of the 2 regression lines (\(P=0.008\)). Similarly, when the NO donor, SNP-induced responses were analyzed the slopes between normotensive and hypertensive groups was also found to be significantly different (\(P=0.041\)).

Moreover, no significant associations were found in any other variables investigated on bradykinin-induced responses (age: \(P=0.617\); gender/male: \(P=0.341\), diabetes mellitus: \(P=0.496\), coronary artery disease: \(P=0.809\), high cholesterol levels: \(P=0.418\), \(\beta\)-blockers: \(P=0.477\), ACE-inhibitors: \(P=0.269\), Nitrates: \(P=0.640\), Diuretics: \(P=0.670\), Lipid lowering drugs: \(P=0.444\), and also on SNP-induced responses (age: \(P=0.406\); gender/male: \(P=0.612\), diabetes mellitus: \(P=0.147\), coronary artery disease: \(P=0.793\), high cholesterol levels: \(P=0.340\), \(\beta\)-blockers: \(P=0.516\), ACE-inhibitors: \(P=0.934\), Nitrates: \(P=0.739\), Diuretics: \(P=0.410\), Lipid lowering drugs: \(P=0.115\)).

**Brachial Artery Dilations to Hyperemic Flow (FMD) and Nitroglycerin (NTG)**

To reveal that alterations in the vasomotor function of coronary microvessels are mirrored in that of large peripheral conduit arteries, in a separate group of patients with documented hypertension (\(n=55\)), brachial artery dilations were also assessed by high-resolution ultrasound. Patient demographics are summarized in Table 2. In the study population the mean value of FMD was 5.0±0.4%, whereas the mean value of NTG-induced dilation was 16.1±6.6%. Interestingly, when patients were divided to lean (BMI <25), overweight (BMI between 25 and 30) and obese subgroups (BMI >30), FMD- and NTG-induced dilations of brachial artery (Figure 4a) were significantly enhanced between the lean and obese subjects (FMD, lean versus obese: \(P=0.026\), overweight versus obese: \(P=0.011\) and NTG, lean versus obese: \(P=0.023\)). Correspondingly, Pearson correlation revealed a significant positive correlation between BMI and FMD (Figure 3b) and also between BMI- and NTG-induced (Figure 4b) brachial artery dilations. Vessel size and hyperemia were similar in lean, overweight, and obese patients, thus it can be assumed that the stimulus for FMD was similar in the groups studied. Also, images taken in lean and obese subjects were comparable in quality and resolution making feasible for the comparison of patients according to their body weight (supplemental Figure I, available online at http://atvb.ahajournals.org).

In addition to a significant positive correlation between BMI and brachial artery dilations, a significant negative correlation was found between FMD and age (\(r=-0.32\), \(P=0.021\)), but not between NTG-induced dilations and age (\(r=-0.15\), \(P=0.285\)). Thus, this variable was then used in the multiple regression analysis, as a covariate. Correlations between BMI- and FMD- and also BMI- and NTG-induced dilations, however, remained significant after adjusting for
age (FMD versus BMI: \(P = 0.047\) and NTG versus BMI: \(P = 0.007\)). No significant correlations were found in any other variables investigated on FMD (gender/male: \(P = 0.196\), diabetes mellitus: \(P = 0.200\), coronary artery disease: \(P = 0.499\), high cholesterol levels: \(P = 0.611\), \(\beta\)-blockers: \(P = 0.428\), ACE-inhibitors: \(P = 0.317\), Diuretics: \(P = 0.230\), Lipid lowering drugs: \(P = 0.922\)) and also on NTG-induced brachial artery dilations (gender/male: \(P = 0.245\), diabetes mellitus: \(P = 0.245\), coronary artery disease: \(P = 0.108\), high cholesterol levels: \(P = 0.541\), \(\beta\)-blockers: \(P = 0.619\), ACE-inhibitors: \(P = 0.980\), Diuretics: \(P = 0.566\), lipid lowering drugs: \(P = 0.570\)).

**Discussion**

The novel findings of this study are that in isolated coronary arterioles of hypertensive patients, bradykinin- and the NO donor, SNP-induced dilations were augmented in obese individuals compared with those of lean subjects. Alterations in coronary arterioles were mirrored in large peripheral arteries in that there was a positive correlation between the FMD- and NTG-induced dilations of the brachial artery and BMI among hypertensive patients. Collectively, these findings suggest an important adaptation of the dilator function of coronary microvessels and peripheral arteries to the simultaneous presence of obesity and hypertension.

Although it has been suggested that obesity increases the risk for developing coronary heart disease\(^3,4\) the impact of obesity on coronary vasomotor function, especially in the presence of other diseases, such as hypertension, is poorly understood. There is a general agreement that in hypertension vascular dysfunction develops, which may contribute to the enhanced cardiac risk in these individuals.\(^11,12\) Vasomotor dysfunction is primarily characterized by reduced endothelium-dependent dilations both in large conduit vessels and resistance arteries.\(^7,12\) In subjects with hypertension a reduced brachial artery relaxation to hyperemic flow (FMD) has been demonstrated previously.\(^11\) In obesity, an early development of vascular dysfunction has also been described.\(^13,23\) For instance, in obese children with risk for atherosclerosis impaired FMD- and NTG-induced relaxation of brachial arteries was reported recently.\(^14\) These observations sug-
gested that both hypertension and obesity are associated with impaired conduit artery relaxations. Because of the close correlation between coronary arterial function and brachial artery relaxation, we hypothesized that simultaneous presence of hypertension and obesity has detrimental effect on coronary arteriolar vasomotor function.

Thus, we studied the responses of coronary arterioles, isolated from the heart of patients who underwent cardiac surgery, to bradykinin and SNP, whose mechanism of actions are well known. In isolated coronary arterioles we have found no differences in the magnitude of bradykinin- and SNP-induced dilations between normotensive and hypertensive patients (Figure 1). Because there were no major differences between the 2 groups in other investigated variables (Table 1), it seemed that the presence of hypertension has no significant impact on coronary arteriolar dilations in this study population. However, when the magnitudes of coronary dilations were investigated in lean versus obese subjects, marked differences could be revealed between the normotensive and hypertensive groups. We have found that in normotensive patients, obesity was associated with reduction of agonist-induced coronary dilations (both bradykinin-induced, endothelium-dependent and also SNP-evoked, endothelium-independent). Also, we observed that in lean patients presence of hypertension was associated with impairment of coronary dilations (Figure 1c and 1d). These observations are in line with the large body of literature, suggesting detrimental effects of hypertension11 and obesity12,23 on vasomotor responses. However, the important and new finding of this study that in the simultaneous presence of hypertension and obesity, coronary arteriolar dilations to bradykinin and the NO-donor, SNP were markedly enhanced (Figure 1c and 1d) and also dilations were positively correlated with BMI in these hypertensive patients (Figure 2). These findings may explain the lack of differences in the averaged data regarding coronary dilations between normotensive and hypertensive groups (Figure 1a and 1b), in which patients with various body weights were lumped together. The key impact of body weight affecting coronary dilator responses were also substantiated by the results showing no significant impact of other potential factors, such as age, gender, and existing comorbidities, and medications. It should be noted that when agonist-induced coronary arteriolar dilations were evaluated by ANOVA, a possible interaction between hypertension and obesity was also indicated. Given that, although our results suggest a potential impact of obesity on coronary arteriolar dilations in hypertensive patients, conclusion in regard to the independent effect of obesity and hypertension affecting vasomotor function has limitations.

Previous studies also demonstrated a close association between the vasomotor function of coronary arterioles and peripheral brachial artery dilation to hyperemic flow (FMD).15,16 Thus, in the present study we also aimed to test the impact of simultaneous presence of obesity and hypertension on the vasomotor responses of the brachial artery. Particularly, we were interested whether the findings obtained in coronary microvessels are somewhat mirrored in those of large conductance vessels in hypertensive patients. In this study we have found that both FMD- and NTG-induced dilations of brachial artery were significantly elevated in obese hypertensive patients, when compared with lean hypertensive subjects (Figures 3 and 4). Correspondingly, a positive correlation between BMI- and FMD- and also BMI- and NTG-induced brachial artery dilation in hypertensive patients was found (Figures 3 and 4), similar to those of findings obtained in isolated coronary arterioles (Figures 1 and 2). Correlations between BMI- and FMD- and also BMI- and NTG-induced brachial dilations remained significant even after adjusting for age, which variable was found to be negatively correlated with FMD of the brachial artery. No significant associations were found when the magnitude of FMD and NTG-induced brachial artery relaxations were compared with other variables, such as gender, comorbidities, and medications.

It should be noted that in this study there was a predominance of overweight and obese-hypertensive patients, when compared with those of lean-hypertensive individuals. This was taken into the account, when comparison of the results obtained from subgroups with different numbers was accomplished by ANOVA. Moreover, the impact of BMI, being a continuous variable, was also evaluated by Pearson correlation, to validate the key impact of overweight and obesity on vasomotor responses. Although these analyses demonstrated a positive, rather than a negative (as one would expect), association between BMI- and FMD- and also between BMI- and NTG-induced brachial artery dilations, still, the possible influence of the predominance of overweight and obese subjects cannot be entirely excluded, which may limit the final conclusions drawn by this study. Taken together, our present findings strongly suggest that in hypertensive patients overweight and obesity is in close association with enhanced/adaptive arterial dilations not only in the coronary microvessels but also in the peripheral conduit arteries.

The mechanisms responsible for the observed effect of obesity on vascular function of hypertensive patients are likely to be complex. Bradykinin-induced coronary arteriolar dilations and FMD of the brachial artery are considered being partly dependent on endothelium-derived relaxing factors, such as NO, whereas SNP- and NTG-induced dilations are dependent on the responsiveness of vascular smooth muscle to NO.25,26 Because both endothelium-dependent (NO agonist)- and -independent (NO donor)-induced coronary arteriolar and brachial artery dilations were enhanced in obese hypertensive patients, it is likely that primarily the enhanced sensitivity of vascular smooth muscle cells to NO is responsible for the observed alterations. This assumption is further supported by the findings showing potential limitations of the results obtained with the endothelium-dependent dilator, bradykinin in coronary microvessels. Nevertheless, the possible involvement of endothelial mechanisms cannot be completely excluded. Interestingly, recent studies elucidating alterations in vasomotor function in animal models of obesity also demonstrated preserved or even enhanced coronary arterial dilations, although the exact mechanisms remained obscure.18,19 A very recent study has reported that in patients with morbid obesity, rapid weight reduction is associated with reduction of NO synthesis.27 These investigations support our present findings that in certain conditions,
obesity could activate adaptive vascular mechanisms, among others by increasing the sensitivity of vascular smooth muscle to NO, aiming to maintain/enhance vasodilatory function of arterial vessels.

At this time, however, one can only speculate regarding the physiological or clinical relevance of the present observation. The hallmark of essential hypertension is known to be an increased total peripheral resistance. With the progression of hypertensive cardiovascular disease, cardiac output begins to fall, and total peripheral resistance becomes more elevated. Conversely, any increase in body mass (muscular or adipose tissue) requires a higher cardiac output and expanded intravascular volume to meet the elevated metabolic requirements. An enhanced dilatory function of coronary arterioles may reflect increased coronary blood flow and metabolism caused by hyperdynamic circulation early in hypertension and obesity. Interestingly, in early studies it has been postulated that obesity may protect a given patient from the deleterious effect of hypertension by decreasing hypertensive target organ damage. Furthermore, the increased NO sensitivity of coronary arterioles in obese and hypertensive individuals, revealed in the present study, might have beneficial effects regarding to the efficacy of nitrate therapy, as well as the prevalence of nitrate tolerance in this particular patient population, which has yet to be elucidated.

Taken together, the present study is the first to show a close association between obesity and the magnitude of dilations of coronary microvessels and peripheral conduit arteries of hypertensive patients. Obesity seems to activate intrinsic vascular mechanisms, such as increased NO sensitivity, implying an important functional adaptation of arterial vessels in the coronary and peripheral circulation to the simultaneous presence of obesity and hypertension.

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Disclosures
None.

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Supplementary Figure 1, Representative images obtained from lean (Panels a and b) and obese patients (Panels c and d) at baseline (Panels a and c) and after nitroglycerin (NTG) administrations (Panels b and d), showing a comparable quality and resolution of the images in lean and obese subjects making feasible for the comparison of patients according to their body weight.