

# **MECHANISMS OF VASCULAR ADAPTATION TO OBESITY**

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**Ph.D. THESIS**

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**List of full papers related to the subject of the thesis:**

- I. Fulop T, **Jebelovszki E**, Erdei N, Szerafin T, Forster T, Edes I, Koller A, Bagi Z. Adaptation of vasomotor function of human coronary arterioles to the simultaneous presence of obesity and hypertension. *Arterioscler Thromb Vasc Biol* 27: 2348-2354, 2007. **(IF:7,221)**
  
- II. **Jebelovszki E**, Kiraly C, Erdei N, Feher A, Pasztor ET, Rutkai I, Forster T, Edes I, Koller A, Bagi Z. High-fat diet-induced obesity leads to increased NO sensitivity of rat coronary arterioles: role of soluble guanylate cyclase activation. *Am J Physiol Heart Circ Physiol* 294: H2558-2564, 2008. **(IF:3,643)**
  
- III. **Jebelovszki É**, Király I, †Török L, Deák G, Varga A, Pajor L, Forster T. A kari verőér rugalmasságának mérése merevedési zavarban. *Magyar Urológia* 20: 13-17, 2008.

## List of Abbreviations

ACE - angiotensin converting enzyme  
ACh - acetylcholine  
ANOVA – analysis of variance  
BMI - body mass index  
cGMP - cyclic GMP  
DAB - diaminobenzidine (brown product)  
eNOS – endothelial nitric oxide synthase  
FMD - flow-mediated dilatation  
L-NAME - *N*<sup>o</sup>-nitro-L-arginine-methyl-ester  
MetS - Metabolic syndrome  
NO - nitric oxide  
NTG - nitroglycerin  
ODQ - oxadiazolo-quinoline  
PBS - physiological salt solution  
SD - standard deviation  
SEM - standard error of the mean  
sGC - soluble guanylate cyclase  
SNP - sodium nitroprusside  
ZDF - Zucker diabetic fatty rats

## 1. SUMMARY

Metabolic syndrome (MetS) is associated with clustering of cardiovascular risk factors in individuals that may greatly increase their risk of developing coronary artery disease. Obesity and related metabolic dysfunction are the driving force in the prevalence of MetS. It is believed that obesity has detrimental effects on cardiovascular function, but its overall impact on the vasomotor regulation of small coronary arteries is still debated. We aimed to examine the impact of obesity on the vasomotor function of large conduit vessels and small coronary arterioles. We have found that in the brachial artery there was a positive correlation between flow-mediated (FMD)- and nitroglycerin (NTG)-induced dilations and body mass index (BMI) in obese patients.

In animal model of diet-induced obesity, we demonstrated that due to the activation of soluble guanylate cyclase the sensitivity of vascular smooth muscle cells to nitric oxide is enhanced, which contributes to the enhanced coronary arteriolar dilations to nitric oxide donors.

Our data indicate that in obesity arteries adapt to hemodynamic changes via up-regulating cellular mechanism(s) intrinsic to the vascular wall. A better understanding of mechanisms that may contribute vascular adaptation may provide insight into the sequence of pathological events in obesity and may allow the harnessing of these effects for therapeutic purposes.

## **2. BACKGROUND**

Metabolic syndrome (MetS) is associated with a clustering of cardiovascular risk factors in individuals that may greatly increase their risk of developing ischemic heart disease and heart failure. Abnormalities in the vasomotor function of the coronary microvessels occurs in MetS; and in some instances these abnormalities represent important markers of risk or may even contribute to the pathogenesis of myocardial dysfunction. Obesity and its related metabolic dysfunction are the driving force in the prevalence of MetS and the development of type 2 diabetes. The coronary microcirculation is currently being therapeutically targeted aiming to prevent or delay the development of cardiac contractile dysfunction, heart failure and ischemic heart disease, which remains the major challenge in reducing morbidity and mortality in patients with MetS.

### **2.a. Progression of coronary vasomotor dysfunction in MetS**

Although acute and chronic ischemic syndromes are commonly due to coronary flow-limiting atherosclerotic plaques in epicardial coronary arteries, about 10 to 20% of patients with cardiac symptoms undergoing cardiac catheterization are found to have normal coronary angiograms (11). It has been demonstrated that despite the presence of angiographically normal coronary arteries coronary flow reserve is reduced in diabetic patients (57). Thus, it has been suggested that epicardial atherosclerosis may not be the only underlying pathology resulting in abnormal coronary flow reserve in diabetic patients (55, 57). In type 2 diabetic patients impaired acetylcholine (ACh)-induced, endothelium-dependent relaxation of brachial artery (18) and forearm resistance vessels has been reported earlier (26, 49, 70). Performing quantitative angiography, Nitenberg et al has demonstrated impaired coronary dilation using the cold pressor test in type 2 diabetic patients with angiographically normal coronary arteries (56). Using similar methodology, Kaneda et al have performed a study, in which 165 patients underwent intracoronary injection of ACh and found that diabetes was the strongest predictor for ACh-induced vasospasm, as an indicator of endothelial dysfunction (37). Moreover, in coronary arterioles isolated from the heart of diabetic patients, Miura et al demonstrated an impaired hypoxia and ATP-dependent, potassium channel-mediated vasodilation (50). Thus, strong evidence indicates that in MetS development of diabetes mellitus is associated with impaired vasodilator responses of both peripheral and coronary microvessels.

Data obtained in animal models of MetS also indicate that presence of fasting hyperglycemia, hence presence of manifest diabetes, impairs coronary vasodilation to agonists and to increases in intraluminal flow (10, 13, 15, 47, 65). Oltman et al have demonstrated that in Zucker diabetic fatty (ZDF) rats coronary arteriolar dilation to ACh is diminished, whereas dilation in prediabetic, younger (8-12 weeks old) animals are preserved (59). Of note, in pre-diabetic obese Zucker rats or in animals fed a high fat diet, with mild elevation of fasting glucose levels, impaired vasodilator function has been reported in vessels from the mesentery (59), cerebral (21, 22) and skeletal muscle vascular beds (19, 24). In contrast, in the obese Zucker rats and also in animals fed a high fat diet, recent studies found preserved (31, 34, 41) or even augmented coronary dilations (60). Thus, it seems that during the progression of MetS while coronary vasomotor function is protected before the development of type 2 diabetes, peripheral microvessels exhibit impaired vasomotor regulation.

To address this discrepancy it has been posited that, as compared to the vascular beds of the periphery, coronary microvessels are more “resistant” to development of vasomotor dysfunction (40). This implies, that coronary vessels either have efficient mechanisms, which protect their vasomotor function, or that coronary vessels have mechanisms that can actively compensate for the loss of vasomotor pathways, as metabolic disease progresses (25, 68). Since in the coronary circulation oxygen extraction is near maximal (71), any impairment in arteriolar dilator function could have significant consequences on myocardial perfusion. As described by Chilian, the coronary circulation matches blood flow with oxygen requirements by coordinating the resistances within different-sized vascular beds, each governed by distinct regulatory mechanisms (14, 36). Such integration in the coronary circulation is believed to be advantageous because the system does not rely on a single mechanism of control, i.e. myogenic, flow or metabolic regulation of vascular resistance (51). An integration of vasomotor regulatory systems in the coronary circulation seems especially important in obesity and MetS, conditions in which metabolic and hemodynamic changes require adaptation of coronary vasomotor regulation.

In MetS there could be several factors that can be implicated necessitating adaptation of coronary vessels. In MetS, the impact of these pathological factors is difficult to discern owing to the close interrelationships between obesity, insulin resistance, type 2 diabetes, hypertension and other known and as yet unidentified pathological factors (16, 17). Yet, several previous and recent studies raised the possibility that the early adaptation of the coronary circulation can be attributed specifically to obesity and/or obesity-related changes in

metabolic and hemodynamic regulation. On the other hand, adaptive vasomotor responses in the coronary circulation may decline as MetS progresses and other co-morbid diseases develop, such as, severe insulin resistance, hypertension and fasting hyperglycemia (diabetes). This may lead to limited vasomotor function (both dilator and constrictor functions can be diminished at advanced state of a disease) of coronary microvessels that are primarily responsible for adjusting cardiac perfusion to actual metabolic demand.

## **2.b. Obesity and cardiovascular regulation**

We make no attempt to provide a detailed description of the impact of obesity on complex hemodynamic regulation or the functional and structural changes of the heart but refer to a comprehensive recent review (1). Of particular importance, is the widely accepted view that obesity is independently associated with left ventricular hypertrophy. A large body of evidence indicates that an increase of left ventricular mass, in the long term, leads to diastolic and systolic cardiac contractile dysfunction in obese patients (1). It has been also posited that in “uncomplicated” (lack of co-morbid conditions such as hypertension, diabetes etc.) obesity associated increases in left ventricular mass can be appropriate for body size (33). Thus, early “physiological” adaptation of cardiac function can be envisioned, which will accommodate for the higher hemodynamic and metabolic demand in obesity. It is known that any increase in body mass (muscular or adipose tissue) requires a higher cardiac output and expanded intravascular volume to meet the elevated metabolic requirements (43). It is also believed that obesity is associated with a hyperdynamic circulation and increased cardiac output (43). Correspondingly, a study by Jern et al (35) has demonstrated that cardiac output and stroke volume were positively related to body mass index (BMI), but inversely to waist/hip ratio. They also found that total peripheral resistance was inversely correlated to BMI, whereas high waist/hip ratio was associated with higher systemic vascular resistance (35). This implies that increased BMI can be associated with increased cardiac output and lower peripheral vascular resistance, but visceral obesity, which is the case in many obese patients, is associated with lower cardiac output and higher total peripheral resistance. Whether these changes can be attributed to an altered cardiac structure or contractile dysfunction or whether they can be related to alterations in the function of coronary and peripheral resistance vessels is not known. The impact of obesity on complex cardiovascular regulation over the course of progression of MetS clearly requires further mechanistic investigations.

## **2.c. Impact of obesity on vasodilator function**

Morphological changes in microvessels are quite rare in obesity prior to the development of hyperglycemia. Obesity-related pathological alterations, including atherogenic dyslipidemia, insulin resistance and hyperinsulinemia are believed to impair the vasomotor function of small arteries. However, blood flow to the various organs systems is rarely impaired in obesity, unless atherosclerosis of the arteries develops. Throughout life, organs receive normal or even greater than normal blood flow in obese subjects (29). Yet, convincing evidence of the impact of obesity on vasomotor regulation of coronary microvessels is lacking at present. Such demonstration is hampered by issues regarding direct investigation of coronary microcirculation both in humans and animal models (14, 61); and also by the impact of several, combined risk factors present in obesity.

### ***Studies on obese patients***

Central obesity was found to be associated with reduced bradykinin- or hyperemia-induced forearm blood flow (30, 73). It has been shown that obese children already exhibit impaired brachial artery relaxation to hyperemic flow (38). Forearm resistance vessels also exhibited reduced acetylcholine (ACh) and NO-donor (sodium nitroprusside)-induced dilations in obese humans (64). Interestingly, it has been posited that body fat distribution, rather than body weight increase is responsible for the impaired brachial artery dilation (30) and elevation of peripheral vascular resistance in obesity (35), an idea, which is further supported by a theoretical analysis using physiological measurements obtained in obese patients (23).

Only a limited number of studies are available that investigated alterations in vasomotor responses of coronary microvessels in obese patients. Because other studies (2, 69) have demonstrated a close association between coronary vasomotor function and relaxation of brachial artery it was speculated that obesity may also adversely affect coronary dilations. Indeed, myocardial blood flow, as measured by positron emission tomography, was found to be significantly reduced in postmenopausal women with obesity, which was negatively correlated with waist/hip ratio (48). These observations indicated that obesity, especially in the presence of co-morbidities, such as hypertension and diabetes, is not necessarily associated with impaired vasodilator function of coronary microvessels. On the contrary, it is possible that the presence of obesity has potentially a key role in maintaining and augmenting

vasodilator capacity of coronary microvessels. Interestingly, clinical studies on obese patients with coronary heart disease have found an unexpectedly favorable prognosis on acute cardiovascular outcome, with the worst prognosis associated with either underweight or morbidly obese patients (27, 28, 32).

Although obesity is widely accepted as a risk factor for coronary heart disease and heart failure, emerging evidence supports a protective role of obesity once patients have developed cardiovascular disease (28, 32).

### ***Studies in animal models of obesity***

Disturbed regulation of microvascular vasomotor function in animal models of obesity is similar in characteristics that seen in humans. This similarity also applies to the discrepant findings obtained in various vascular beds, in different models of obesity. In the obese, JCR:LA-corpulent rats an impaired endothelium-dependent relaxation of aorta (8) and reduced dilations of mesenteric arteries to ACh (58) have been reported. Reduced mesenteric (53) and skeletal muscle (19) arteriolar dilation to ACh was also found in rats fed a high fat diet. In obese Zucker rats it has been reported that in mesenteric arterioles endothelium-dependent relaxation to ACh was preserved at 20-week old, but was reduced in 32-week old animals (67). In a similar experimental design, Oltman et al have investigated the progression of coronary and mesenteric arterial dysfunction in the Zucker obese rat. They found that coronary arteriolar dilation to ACh was preserved in 16-24 week old animals, but dilations became diminished in 28-36 week old rats, when compared to lean controls (59). Mesenteric arterioles of Zucker rats exhibited relatively maintained dilations to ACh at both ages (59). Katakam et al reported that in 12-week old obese Zucker rats ACh-induced dilations of small coronary arteries was preserved, although they found a reduced vasodilation to insulin (39). These studies concluded that arteriolar dilations in obese animals can be preserved at younger age (i.e. at the early state of disease), but vascular dysfunction progresses and this progression can be at a different rate in different vascular beds, such as in coronary and mesenteric vessels.

More importantly, the early study by Subramanian et al found that relaxation of aorta to ACh is enhanced at ages of both 20 and 32-week old obese Zucker rats animals (67), results similar to those observed earlier by Auguet et al (3). In mice fed a high fat diet, an enhanced endothelium-dependent, hydroxyl radical-induced relaxation in the femoral artery has been also reported (6). Coronary arterioles from female pigs fed a high fat diet exhibited only modest impairment of dilation to bradykinin (31), whereas coronary dilations to ACh

were preserved in the obese Zucker rats (39) and in rats fed a high fat diet (34). More intriguing, Prakash et al have reported that ACh-induced dilations of coronary arterioles from obese Zucker rats were markedly enhanced (more than 25% increase in diameter, when compared to lean animals) (60). These latter observations imply that although coronary dysfunction progresses with obesity, coronary vasodilator function can be preserved or even augmented at early phases of the disease.

Collectively, on the basis of these aforementioned human and animal studies we have raised the hypothesis that vessels (both coronary and peripheral) adapt to obesity by maintaining or enhancing their dilator function to increase blood flow to higher metabolic demand. Emerging evidence indicate that hemodynamic adaptation is not a passive phenomenon, but requires active participation of various cellular pathways at vascular level. The nature of these cellular pathways that are responsible for vascular adaptation to obesity is incompletely understood. Elucidating these cellular mechanisms seems important, not only because they provide insight into the sequence of pathological events in obesity, but also because they could be harnessed for therapeutic purposes.

### 3. AIMS

These aforementioned findings led us to the hypothesis that obesity activates, as yet unknown adaptive mechanisms intrinsic to the vascular wall, aiming to maintain adequate tissue perfusion.

Thus, the aims were:

*1) To investigate the impact of obesity on the endothelium-dependent and –independent dilations of the human brachial artery; and*

*2) To investigate the impact of obesity in animal model (Wistar rat) on coronary arteriolar vasomotor function, to examine the vascular adaptation and the possible cellular mechanisms involved.*

Since nitric oxide (NO) plays an important role in regulating both brachial artery and coronary arteriolar dilations we have focused our investigation on the possible alterations in NO-mediated vasomotor function.

## 4. METHODS

### *4.a. Assessment of brachial artery relaxation*

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. Assessment of brachial artery relaxation was performed in patients ( $N=55$ ), who were recruited locally.

Ultrasound measurements of the brachial artery were performed according to the method described by Celermajer et al (12), using high-resolution ultrasounds 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 mmHg 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 relaxation to hyperemic flow (flow mediated dilation, FMD) and to nitroglycerin (NTG) 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.

### *4.b. Animal model of obesity*

Male Wistar rats ( $N=50$ ) were purchased from Charles River Laboratories. Rats were maintained in the animal care facility with a 12-hour light/dark cycle and were given free access to food and water. Rats were maintained on standard rat chow ( $N=25$ ) or on high-fat diet ( $N=25$ ), (European Union-modified rodent diet with 60% fat, 58Y1, TestDiet, PMI Nutrition International) for 10 weeks (10). All protocols were approved by the Institutional Animal Care and Use Committee.

#### ***4.c. Isolation of rat coronary arterioles.***

With the use of microsurgical instruments and an operating microscope, the second branch of septal artery (~1.5 mm in length) running intramuscularly was isolated and cannulated. The cannulated arteriole was connected with silicone tubing to a pressure servo control system (Living Systems Instrumentation, VT, USA) to set the intraluminal pressure to 80 mmHg. Changes in arteriolar diameter were continuously recorded with a digital camera (CFW1310, Scion Corp, USA), connected to a microscope (Nikon, Eclipse 80i) (2).

#### ***4.d. Assessment coronary arteriolar responses***

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 mmHg. Cumulative concentrations of the endothelium-dependent vasodilator, acetylcholine (ACh, 1 nmol/L – 1  $\mu$ mol/L) were administered to the coronary arterioles from lean and obese rats in the presence and absence of *N*<sup>o</sup>-nitro-L-arginine-methyl-ester (L-NAME; 200  $\mu$ mol/L, for 30 min), an inhibitor of the NO synthase, and changes in diameter were measured. Then, arterioles were incubated with soluble guanylate cyclase (sGC) inhibitor, oxadiazolo-quinoxaline (ODQ, 10  $\mu$ mol/L, for 30 min) and arteriolar responses to ACh were obtained again in the 2 groups. In the separate set of experiments, dilations to cumulative concentrations of NO donor, sodium nitroprusside (SNP, 1 nmol/L – 10  $\mu$ mol/L) was investigated in isolated coronary arterioles of lean and obese rats. Then, arterioles were incubated with ODQ (10  $\mu$ mol/L, for 30 min) and arteriolar responses to NO donor were reassessed. In another series of experiments increasing concentrations of 8-bromo-cGMP, a cell permeable cGMP analog (1 nmol/L – 10  $\mu$ mol/L) was administered and changes in diameter were measured. In isolated vessels 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-mmHg intraluminal pressure in a Ca<sup>2+</sup>-free medium.

#### ***4.e. cGMP immunocytochemistry***

The sGC activity was detected in coronary arterioles through the identification of basal and NO donor-stimulated increases in cGMP immunoreactivity, by using antibody against cGMP, similarly as it was described previously (12). Briefly, the left ventricle including the

coronary arteriole was embedded and frozen in optimal cutting temperature compound (Tissue Tek, Electron Microscopy Sciences). Unfixed consecutive sections (10- $\mu\text{m}$  thick) were transferred to a solution containing 1  $\mu\text{M}$  SNP in physiological salt solution (PBS) and incubated for 15 min at 37 °C. Other sections remained in PBS solution during the 15-min incubation period and served as unstimulated controls. Sections were then fixed with acetone and immunolabeled with a monoclonal anti-cGMP primary antibody (dilution 1:2000, Sigma Inc). Immunostainings were visualized by using avidin-biotin horseradish peroxidase visualization system (Vectastain kit, Vector Laboratories), stained with diaminobenzidine (DAB). For nonspecific binding, the primary antibody was omitted. Images of the sections were collected with a digital camera (CFW 1310C, Scion Corp) connected to a Nikon Eclipse 80 microscope. For semi quantitative analysis of the cGMP immunoreactivity, in defined areas of the arteriolar wall (6 separate regions in each arteriole with diameter of ~100-150  $\mu\text{m}$ ) the amount of the brown product (DAB) was estimated by measuring optical density using the NIH Image software. Background (absence of the first antibody) subtracted, averaged optical density was then calculated and compared in coronary arterioles of lean and obese rats.

#### ***4.f. cGMP ELISA***

The sGC activity was detected in the carotid artery through the identification of basal and NO donor, SNP-stimulated increases in cGMP levels, which was measured with commercially available ELISA kit (Assay Design Inc, Ann Arbor, USA) following the instructions by the manufacturer.

#### ***4.g. Immunoblots***

Single coronary arteries (one vessel from each animal) were dissected from the hearts of lean and obese rats, cleared of connective tissue and briefly rinsed in ice-cold PBS. After the addition of 20  $\mu\text{l}$  of Laemmli sample buffer (from Sigma Inc.) tissues were homogenized. Immunoblot analysis was carried out as described before (3). The antibodies used for detection of protein expression (anti-eNOS IgG and anti-sGC  $\beta$ 1 subunit IgG) were obtained from Sigma Inc. Anti- $\beta$ -actin IgG obtained from Abcam Inc was used as loading control. Signals were revealed with chemiluminescence and visualized autoradiographically. Optical density of bands was quantified and normalized for  $\beta$ -actin by using NIH Image software.

#### ***4.h. Statistical analysis***

Data were stored and analyzed with the NCSS statistical software (Kaysville, Utah, USA). Test selection was based after evaluating the variables for normal distribution, employing the Kolmogorov-Smirnov test. In the human studies, testing differences of different variables between groups was accomplished by two-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. Since 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 two-way ANOVA was performed. 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 calculated. In the animal studies Statistical analyses were performed using GraphPad Prism Software (San Diego California USA) by two-way repeated-measures ANOVA followed by Tukey's posthoc test or Student's t-test as appropriate. Data are expressed as means±S.E.M.  $P<0.05$  was considered statistically significant.

## 5. RESULTS

### 5.a. Brachial artery relaxation to hyperemic flow and nitroglycerin in patients with hypertension

In order to reveal the impact of obesity on vasomotor function brachial artery dilations were assessed by high resolution ultrasound.

Patient demographics are summarized in Table 1.

**Table 1. Demography of Patients Underwent Brachial Artery Ultrasound Investigation**

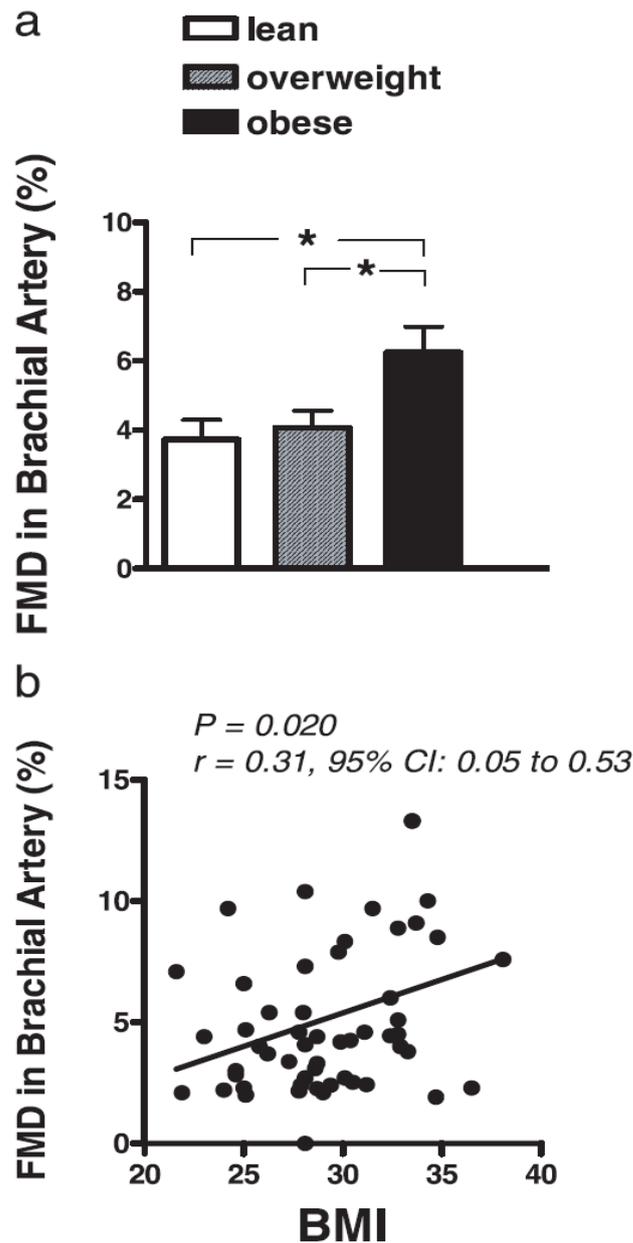
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<b>Characteristics N=55</b>	
Sex, male (%)	34 (61.8)
Age (years)	59±11
Systolic Blood Pressure (mmHg)	153±15
Diastolic Blood Pressure (mmHg)	93±10
Body mass index (BMI)	29±4
<b>Underlying disease N, (%)</b>	
Diabetes Mellitus	20 (36.4)
Hypercholesterolemia	41 (77.4)
Coronary artery disease	9 (16.3)
Previous myocardial infarction	5 (9.4)
<b>Medications N, (%)</b>	
β-blockers	26 (47.3)
ACE inhibitor	40 (72.7)
Diuretics	15 (27.3)
Nitrates	1 (1.8)
Lipid lowering	23 (41.8)

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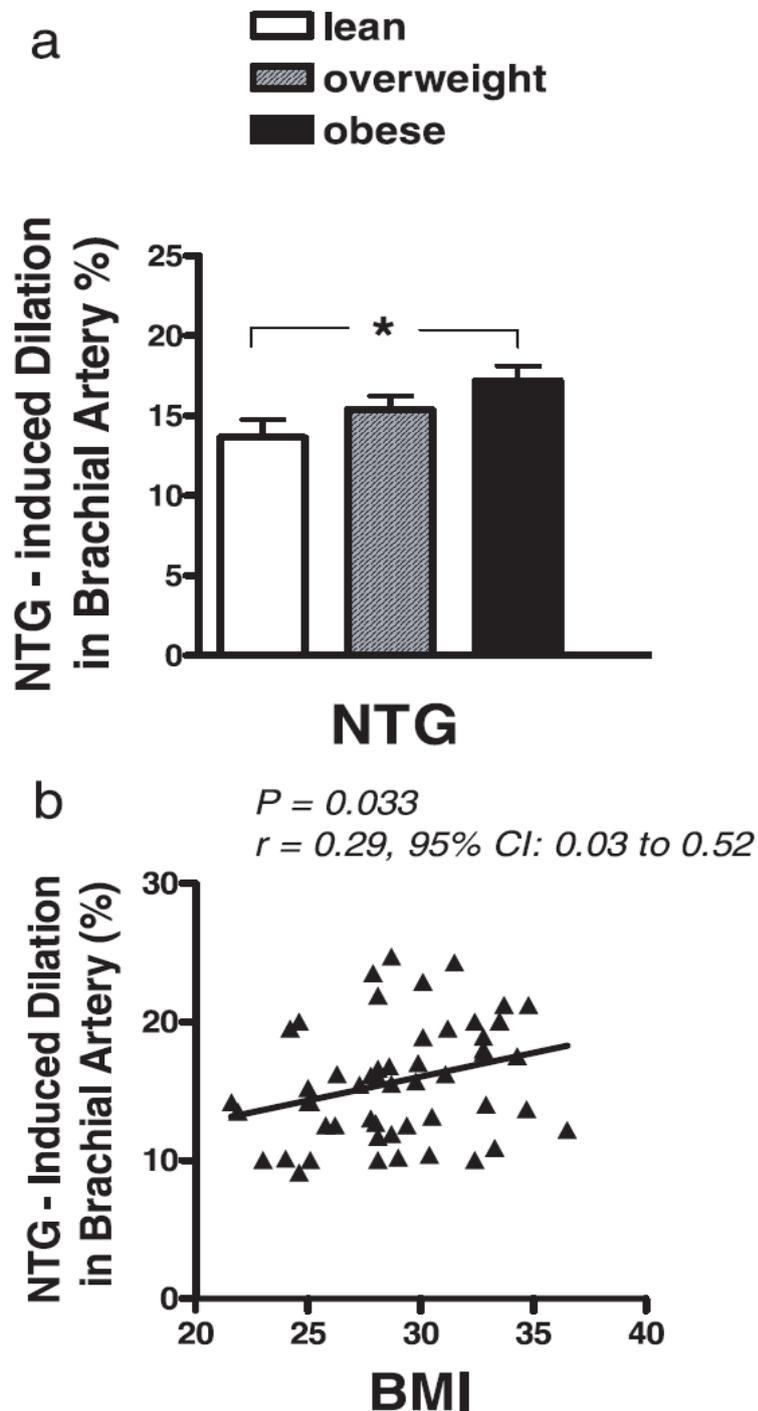
Data are mean±SD. *N* indicates the number of patients studied.

In the study population the mean value of FMD was  $5.0 \pm 0.4\%$ , whereas the mean value of NTG-induced dilation was  $16.1 \pm 0.6\%$ . Interestingly, when patients were divided to lean (BMI < 25), overweight (BMI between 25 and 30) and obese subgroups (BMI > 30), FMD (Figure 1a) and NTG-induced dilations of brachial artery (Figure 2a) were significantly enhanced between the lean and obese subjects (FMD, lean vs. obese:  $P=0.026$ , overweight vs. obese:  $P=0.011$  and NTG, lean vs. obese:  $P=0.023$ ).



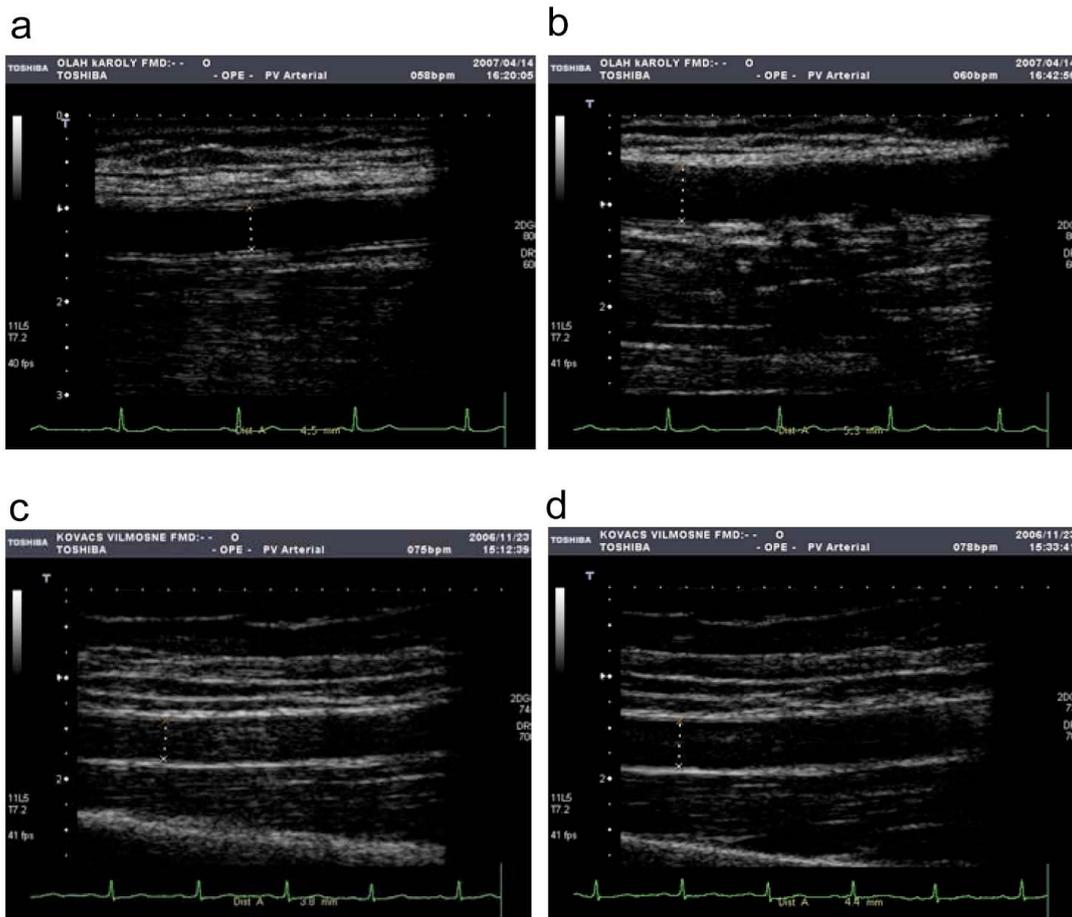
**Figure 1, Panel a:** Summary data of flow-mediated dilation (FMD) of the brachial artery in lean ( $N=11$ ), overweight ( $N=21$ ) and obese ( $N=23$ ) patients with hypertension. Data are mean  $\pm$  SEM. \*,  $P < 0.05$ , **Panel b:** Pearson correlation between FMD and BMI in hypertensive individuals ( $N=55$ , regression line is included in the Figure).

Correspondingly, Pearson correlation revealed a significant, positive correlation between BMI and FMD (Figure 1b) and also between BMI and NTG-induced (Figure 2b) brachial artery dilations.



**Figure 2, Panel a:** Summary data of nitroglycerin (NTG)-induced brachial artery dilations in lean ( $N=11$ ), overweight ( $N=21$ ) and obese ( $N=23$ ) patients with hypertension. Data are mean $\pm$ SEM. \*,  $P<0.05$ , **Panel b:** Pearson correlation between NTG-induced dilations and BMI in hypertensive individuals ( $N=55$ , regression line is included in the Figure).

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 (Figure 3).



**Figure 3,** *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.*

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 vs. BMI:  $P=0.047$  and NTG vs. 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:  $P=0.415$ , 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:  $p=0.570$ ).

### 5.b. Results in animal model of obesity

After commencing of high fat diet for 10 weeks the body weight, serum insulin, glucose, and total cholesterol levels of rats became significantly greater, when compared to those of rats fed the standard diet (Table 2). Also, we have found that the C-reactive protein levels were similar in the two groups of animals (Table 2). It should be noted that we have found a significant elevation in fasting glucose levels in this model of diet-induced obesity. However, the glucose levels were only slightly elevated in obese rats (Table 2), as compared to other animal models of type 2 diabetes, such as the db/db mice (2) or the diabetic Zucker rats (13), in which animals have about 4-times higher fasting glucose levels.

**Table 2: Characteristic of rats after 10 weeks of normal diet (lean) and high fat diet (obese)**

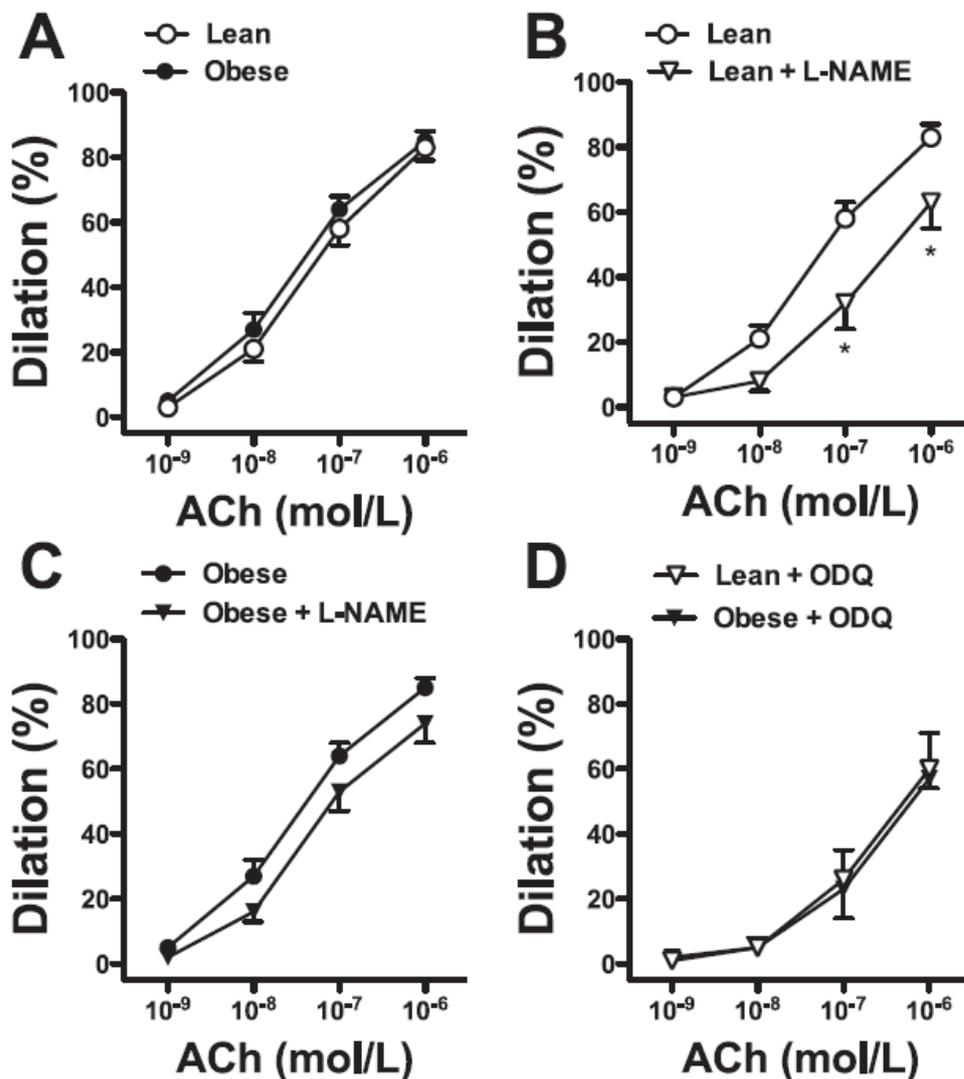
	<b>Lean (n=20)</b>	<b>Obese (n=20)</b>
body weight (g)	376±16	569±17 *
serum glucose (mmol/L)	6.5±0.3	9.6±0.7*
serum insulin (pmol/L)	91±11	221±19*
serum total cholesterol (mmol/L)	1.20±0.08	1.62±0.07*
C-reactive protein (mg/L)	0.12±0.01	0.14±0.01

Data are mean±SEM. \* indicate significant difference,  $p < 0.05$

#### ***Coronary arteriolar responses to ACh.***

In coronary arterioles isolated from lean and obese rats there were no significant differences between the spontaneously developed arteriolar tone (86±6  $\mu\text{m}$  and 97±6  $\mu\text{m}$ , respectively) and in the passive arteriolar diameters (in  $\text{Ca}^{2+}$ -free medium, 153±9  $\mu\text{m}$  and 149±7  $\mu\text{m}$ , at 80 mmHg intraluminal pressure, respectively). We have found that

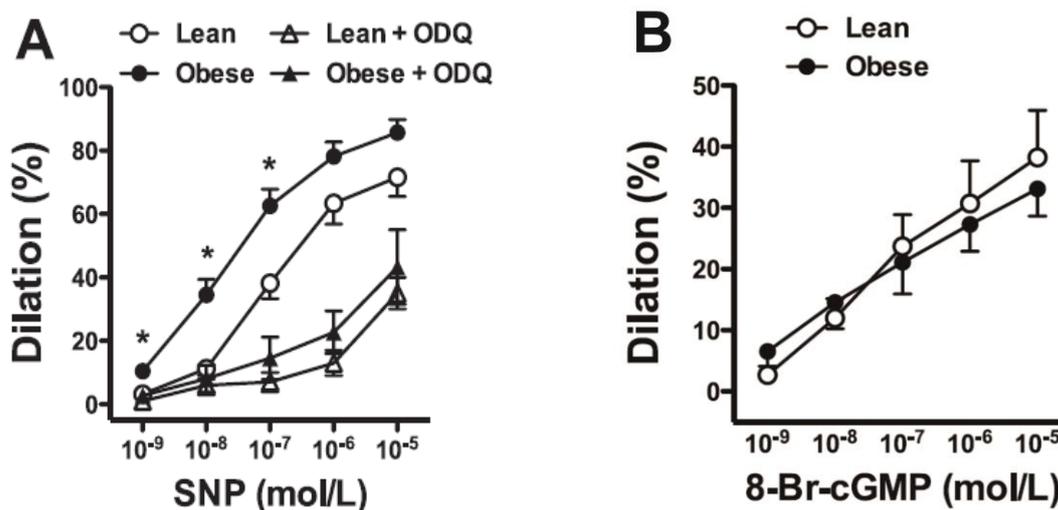
endothelium-dependent dilations to ACh were not significantly different between coronary arterioles of lean and obese rats (Figure 4a). Inhibition of NO synthesis with L-NAME decreased ACh-induced dilation in coronary arterioles isolated from lean animals (Figure 4b), whereas it had not significant effect on ACh-induced responses in arterioles of obese rats (Figure 4c). Administration of ODQ, an inhibitor of soluble guanylate cyclase elicited similar reduction in ACh-induced dilations in coronary arterioles of the two groups of animals (Figure 4d).



**Figure 4**, Changes in diameter of coronary arterioles isolated from lean ( $n=18$ ) and high fat diet-induced obese rats ( $n=19$ ) in response to cumulative concentrations of ACh, before (**Panel a**) and after incubation with L-NAME (**Panel b** and **Panel c**,  $n=9$ ) or oxadiazoloquinoline (ODQ, **Panel d**,  $n=9$ ). Data are mean  $\pm$  S.E.M. Asterisks indicate significant differences ( $P<0.05$ ).

### Coronary arteriolar response to NO donors.

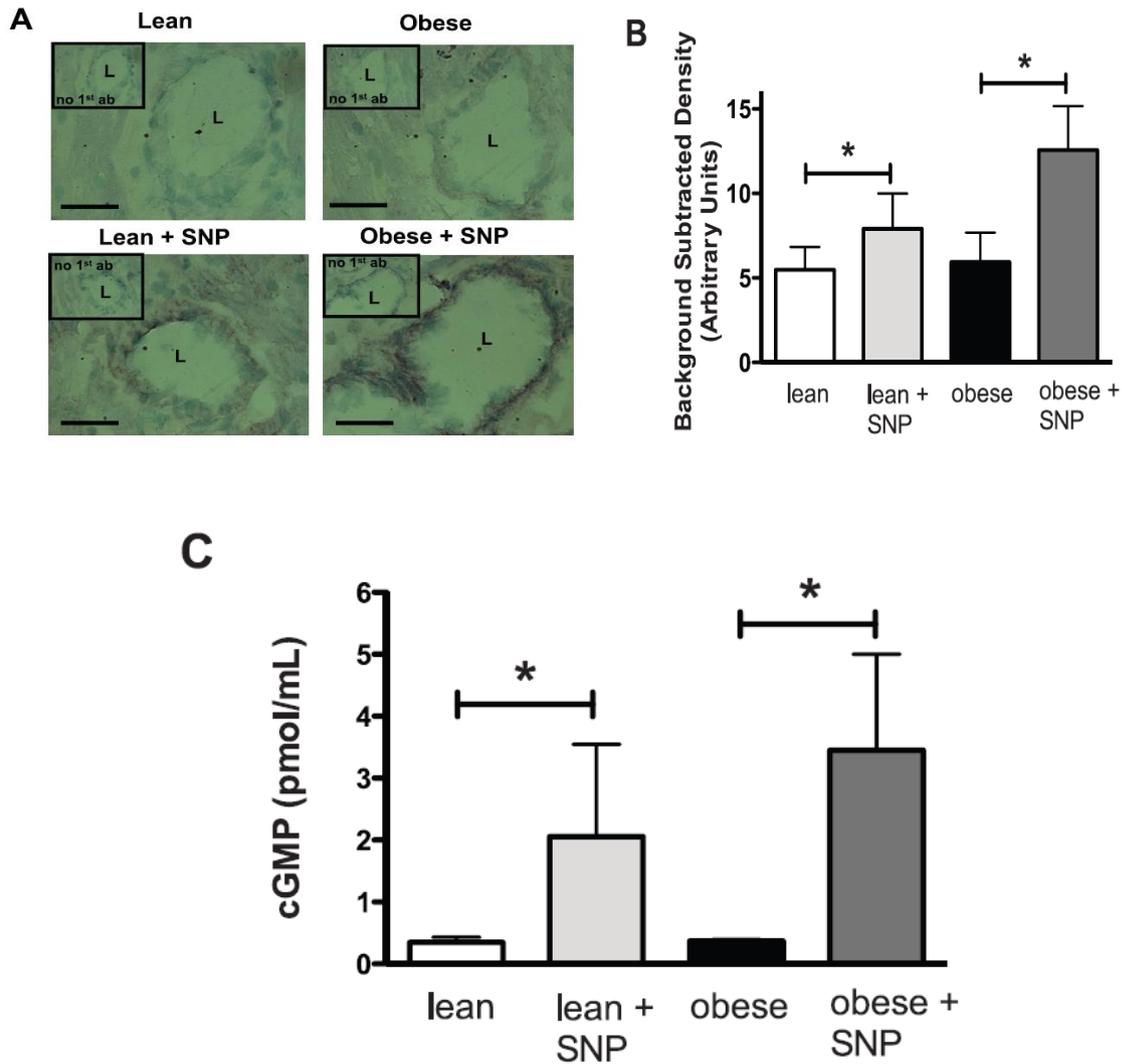
Dilations to the NO donor, sodium nitroprusside (SNP) were also tested and interestingly found to be significantly increased in the arterioles isolated from obese rats, when compared to those of arterioles obtained from lean rats (Figure 5a). SNP-induced dilations were also obtained after administration of ODQ, an inhibitor of soluble guanylate cyclase. ODQ decreased SNP-induced dilations to the same level, thereby eliminating the NO-donor evoked differences in dilations between the two groups (Figure 5a). In separate series of experiments arteriolar responses were obtained to 8-bromo-cGMP, a cell permeable, stable cGMP analog. We found that 8-bromo-cGMP elicited substantial dilations in coronary arterioles, which however were not significantly different in the two groups of vessels (Figure 5b).



**Figure 5,** Changes in diameter of coronary arterioles isolated from lean ( $n=18$ ) and high fat diet-induced obese rats ( $n=19$ ) in response to cumulative concentrations of sodium nitroprusside (SNP, **Panel a**) in the absence and presence of oxadiazolo-quinoxaline (ODQ,  $n=7$ , in each protocol). Changes in diameter of coronary arterioles isolated from lean and obese rats in response to cumulative concentrations of 8-bromo-cGMP (**Panel b**,  $n=7$ ). Data are mean  $\pm$  S.E.M. Asterisks indicate significant differences ( $P<0.05$ ).

### Immunocytochemistry.

Basal and SNP-stimulated cGMP immunoreactivity were detected in native coronary arteriolar section in lean and obese rats. No specific labeling was detected in the section in which the first antibody was omitted. We have found that SNP-stimulated cGMP immunoreactivity was increased in coronary arterioles of lean and obese rats, and the enhancement found to be greater in coronary arterioles of obese rats (Figure 6a and 6b).



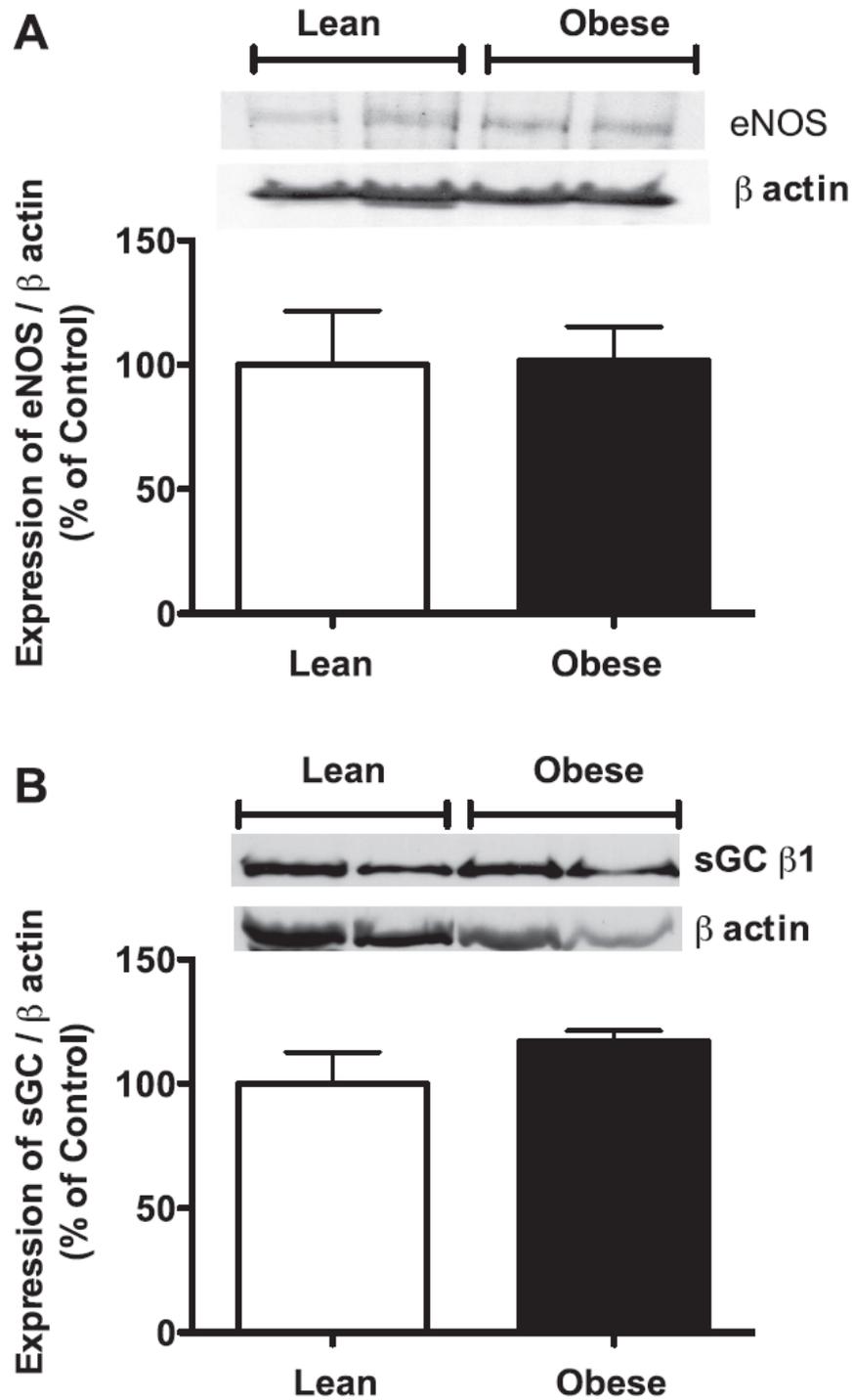
**Figure 6,** Representative photomicrographs of immunocytochemistry (**Panel a**) and summarized data of densitometry analysis of 3 separate experiments (**Panel b**), showing cGMP immunoreactivity (indicated by the brown product) in the coronary arteriolar wall of lean and obese rats, with or without stimulation with SNP. Insets are representing experiments in which the first antibody was omitted (no 1<sup>st</sup> ab). Scale bar, 50 μm, L=arteriolar lumen. **Panel c:** Basal and SNP-stimulated cGMP levels measured with ELISA in carotid arteries isolated from lean (n=5) and obese rats (n=5). Asterisks indicate significant differences (P<0.05).

***cGMP ELISA.***

Basal and SNP-stimulated cGMP levels were directly measured in the carotid artery of lean and obese rats. We have found that basal cGMP levels were similar in vessels from lean and obese rats (Figure 6c). The NO donor, SNP elicited marked increases in cGMP levels in both groups, which tended to be increased in those of vessels from obese rats (Figure 6c).

***Western immunoblots.***

Western blot analysis was performed in single coronary arteries from both lean and obese rats. We have found that there were no significant differences in the eNOS protein expression (Figure 7a) and also in the soluble guanylate cyclase  $\beta$ 1 subunit protein levels in coronary arterioles of lean and obese rats (Figure 7b).



**Figure 7.** Western blot analysis of eNOS (**Panel a**) and soluble guanylate cyclase (sGC)  $\beta$ 1 subunit (**Panel b**) protein expression in single coronary arterioles of lean ( $n=5$ ) and obese ( $n=5$ ) rats. Anti- $\beta$ -actin was used to normalize for loading variations. Bar graphs are summary data of normalized densitometric ratios (1 vessel from each animal was used).

## 6. DISCUSSION

Based on the findings discussed in the Background of this Dissertation we have raised the hypothesis that obesity activates as yet unknown mechanisms intrinsic to the vascular wall, aiming to maintain adequate tissue perfusion during the disease development. Accordingly, the first aim was to investigate the impact of obesity on to endothelium-dependent and –independent dilations of the human brachial artery.

In this set of experiments we have demonstrated that both flow-mediated dilations (FMD) and nitroglycerin (NTG)-induced dilations of brachial artery were significantly elevated in obese patients, when compared to lean hypertensive subjects. Correspondingly, a positive correlation between BMI and FMD and also BMI and NTG-induced brachial artery dilation in hypertensive patients was found. Correlations between BMI and FMD and also BMI and NTG-induced brachial dilations remained significant even after adjusting for age, which variable 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 to other variables, such as gender, co-morbidities and medications. It should be noted that in this study there was a predominance of overweight and obese-hypertensive patients, when compared to those of lean-hypertensive individuals. This was taken into the account, when comparison of the results obtained from subgroups with different *N* 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) associations 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.

The mechanisms responsible for the observed effect of obesity on vascular function are likely to be complex. FMD of the brachial artery are considered being partly dependent on endothelium-derived relaxing factors, such as NO, whereas NTG-induced dilations are depend on the responsiveness of vascular smooth muscle to NO (42, 45). Because, both endothelium-dependent (NO agonist) and -independent (NO donor) induced brachial artery dilations were enhanced in obese patients, it is likely that primarily the enhanced sensitivity of vascular smooth muscle cells to NO is responsible for the observed alterations. Interestingly, recent studies, elucidating alterations in vasomotor function in animal models of obesity also

demonstrated preserved or even enhanced arterial dilations, although the exact mechanisms remained obscure (31, 60). A recent study has reported that in patients with morbid obesity, rapid weight reduction is associated with reduction of NO synthesis (44). 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.

To test this hypothesis the second aim was to investigate the impact of obesity on vasomotor function in animal model of obesity, which allow us a better evaluation of the possible cellular mechanisms involved in enhanced NO sensitivity.

The vascular endothelium produces and secretes numerous compounds that regulate a variety of physiological functions, including vasomotor tone, coagulation, inflammation, permeability and cell adhesion (72). Among others, nitric oxide (NO) is considered to be one of the key molecules in maintaining normal vascular homeostasis and it is a major contributor to maintain adequate coronary microvascular tone (46). Solid evidence indicates that type 2 diabetes is associated with impaired bioavailability of NO both in conduit vessels and resistance arteries (4, 24, 31, 47, 62, 66, 74). Studies have also demonstrated that obese subjects exhibit a reduced NO-mediated, agonist-induced dilation of cerebral, mesenteric, coronary and skeletal muscle microvessels (20, 24, 54). Interestingly, Brandes et al reported that a reduced endothelial NO production by acute administration of an NO synthase inhibitor in wild type mice or chronic deficiency of NO in eNOS knockout mice increase NO sensitivity of vascular smooth muscle cells in response to exogenous NO donors (7). They have proposed that an increased sensitivity of sGC to NO may compensate for the reduced NO synthesis (7). Because administration of exogenous NO decreased sGC activity acutely and over time its protein expression (63), it has been posited that NO may play an important, negative feedback regulatory role on the catalytic activity of the sGC, hence any reduction of NO level may lead to an enhancement of the sensitivity of sGC to NO.

In this set of experiments we have found that in coronary arterioles (active diameter less than 100  $\mu\text{m}$ ) obtained from high fat diet-treated obese rats ACh-induced dilation was essentially preserved. Interestingly, we have found that in coronary arterioles of the obese rats pharmacological inhibition of NOS had no significant effect of ACh-induced dilations, while it reduced those responses in control vessels. This finding suggested either the lack of NO mediation or a reduction of the amount of the NO production in obese animals, which however cannot be detected by measuring diameter changes of arterioles in the presence of a NOS inhibitor. On the other hand, this finding also raised the possibility that in coronary

arterioles of obese rats, mechanisms intrinsic to vascular wall are activated to compensate for the reduced NO availability. In the coronary circulation oxygen extraction is near maximal (33), impairment of arteriolar dilator function could have significant consequence on tissue perfusion, leading to tissue ischemia. It is also known that any increase in body mass (muscular or adipose tissue) requires a higher cardiac output and expanded intravascular volume to meet the elevated metabolic requirements (22). Given that, in obesity coronary resistance vessels should adapt to increased coronary blood flow and metabolic requirements to maintain adequate tissue perfusion during the disease development. Our above-described human study provided evidence for the existence of such adaptation, by showing enhanced dilations to the NO-donor, compared to those of lean individuals.

In the animal model, an attempt was also made to elucidate the possible underlying mechanism contributing to the observed functional adaptation of coronary microvessels in obese rats. Our findings show that in obese rats the sensitivity of coronary arterioles to NO was significantly enhanced, as demonstrated by augmented vasodilations to NO donor, sodium nitroprusside. Interestingly, enhanced dilations of coronary arteries to NO donor, SNP have been also described in female pigs fed with high fat diet, whereas NO-mediated coronary dilations to bradykinin were blunted (36). Collectively, these data suggest that an impaired NO availability in coronary microvessels of obese subjects can be associated with an enhanced NO sensitivity of the coronary arterioles and this mechanism may be responsible for the maintained agonist-induced dilations, also found in the present study.

Increased sensitivity for NO has been already proposed in previous observations in different conditions associated with impaired NO availability. For instance, both acute cessation of endothelial NO production in wild type mice or chronic deficiency of NO in endothelial NO synthase knockout mice increase NO sensitivity of vascular smooth muscle cells in response to nitrovasodilator agents (7). It has been posited that an enhanced sensitivity of the downstream sGC to NO may compensate for the reduced NO availability (7). In this context, it has been also found that acute administration of exogenous NO decreased sGC activity and in long-term its protein expression (30). These findings indicate that NO may play an important, negative feedback regulatory role on the catalytic activity of its effector, sGC, hence any reduction of NO level may lead to an enhancement of the sensitivity of sGC to NO.

To demonstrate the possible involvement of enhanced sGC activation, our studies revealed that inhibition of sGC by ODQ reduced both ACh- and also NO donor (SNP)-induced arteriolar dilations and, importantly, eliminated differences between responses of

coronary arterioles of lean and obese rats. We have also found that the stable cGMP analogue, 8-bromo-cGMP elicited substantial dilations in coronary arterioles, which, however were not significantly different in the two groups of vessels. These findings indicate potential involvement of enhanced sGC activation in mediating enhanced sensitivity of smooth muscle cells for NO in coronary arterioles of obese rats. On the basis of the present findings only minor, if any, role can be ascribed for the contribution of other mechanisms, such as NO/peroxynitrite-dependent, S-glutathiolation-mediated activation of the sarco/endoplasmic reticulum calcium ATPase, which may also lead to an enhanced NO sensitivity, independent of sGC activation, as suggested previously (1). Furthermore, our cytochemistry data suggested a maintained or even enhanced SNP-stimulated cGMP immunoreactivity in the coronary arterioles of obese rats. To quantitate this observation in parallel experiments basal and stimulated cGMP contents were also measured in carotid arteries of lean and obese rats with cGMP ELISA. In this assay we have found no significant differences in the basal cGMP content, but detected elevated cGMP levels in SNP-stimulated vessels of lean and obese rats. Although, results obtained in a conduit vessel cannot be directly extrapolated to those of coronary microvessels, these data are in accordance with immunocytochemistry data obtained in coronary arterioles and suggest maintained or even increased sGC activation in obese animals. The results showing no changes in the protein expression of the sGC  $\beta$ 1 subunit, along with the findings that 8-bromo-cGMP-evoked coronary dilations were similar in lean and obese rats suggest a primary role for enhanced activity of sGC enzyme in coronary arterioles of obese rats.

An intriguing question namely what are the exact origin and nature of factor(s), which may contribute to the activation of sGC is still open. It seems plausible that lack of NO may lead to enhanced sGC sensitivity (7). It has been shown that TNF $\alpha$ -mediated vascular inflammation have important consequences with respect to downstream signaling of NO (25). In this study an attempt was made to estimate the level of systemic inflammation likely to be present in obese rats. To this end we have measured the serum levels of C-reactive protein (CRP), which however found to be comparable in lean and obese animals. This data suggest that in this model a manifest systemic inflammation is unlikely present, although a possible involvement of inflammation localized in the vascular wall cannot be entirely excluded and has yet to be elucidated in future studies.

Collectively, we provided evidence for an enhanced NO sensitivity of coronary arterioles isolated from obese rats fed a high fat diet. We found that the enhanced sensitivity of coronary arterioles to NO was associated with increased activity of sGC in coronary

arterioles, in which a reduced NO bioavailability was also detected. Similar results were obtained in humans, showing that NO donor-induced brachial artery dilations were enhanced in patients with obesity.

Although it seems plausible that the lack of NO release may lead to an enhanced activity of sGC in vascular smooth muscle cells (7), other studies demonstrated that an acute exposure to reactive oxygen species, i.e. H<sub>2</sub>O<sub>2</sub> could also lead to activation of sGC, contributing to the relaxation of the bovine pulmonary artery (9, 75). Moreover, Bauersachs et al have shown that rats with heart failure exhibit increased expression of sGC, which was due to the enhanced vascular superoxide anion production (5). Since obesity is also associated with oxidative stress (19, 59), it is likely that ROS, in addition to their effect in reducing NO availability, may play a role in the activation of sGC, a hypothesis, which has yet to be tested. These data suggest that an impaired endothelial NO availability in coronary arterioles can be associated with an enhanced sensitivity to NO in vascular smooth muscle cells and that this mechanism may lead to compensation of the reduced NO-mediated vascular signaling in obesity. On the other hand, it has been demonstrated that oxidative and nitrosative stress can lead to inactivation of sGC over time (52); thus the question remains to be answered to what extent and how long up-regulation of sGC may compensate for the reduced NO-mediated vascular signaling, as obesity progress.

## 7. SUMMARY AND CLINICAL IMPLICATIONS

Although obesity is widely accepted as a risk factor for coronary heart disease and heart failure, evidence also supports a role for obesity in cardiovascular protection. A growing number of recent reports document a statistically significant survival benefit in obese patients once they have been diagnosed with cardiovascular diseases (27, 28, 32). The conclusion that obesity may both elicit cardiovascular disease and protect from cardiovascular death now clearly requires further investigation at cellular, molecular, and systematic levels.

At this time, we can only speculate regarding the physiological and/or clinical relevance of the present observation. The hallmark of essential hypertension is known to be an increased total peripheral resistance (43). 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 (43). An enhanced dilatory function of coronary arterioles may reflect increased coronary blood flow and metabolism caused by hyperdynamic circulation early in obesity. The increased NO sensitivity of coronary arterioles in obese 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, our studies demonstrated a close association between obesity and the magnitude of dilations of coronary microvessels and peripheral conduit arteries. 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 presence of obesity. Understanding the sequence of pathological events in obesity-related microvascular dysfunction and adaptation might provide a rationale for therapeutic interventions and it might well harness these effects for therapeutic purposes.

## 8. ÖSSZEFOGLALÁS

A metabolikus szindróma egy kardiovaszkuláris rizikófaktorokat, metabolikus változásokat magába foglaló komplex eltérés, amely jelentősen megnöveli a szívelégtelenség, és az ischaemias szívbetegség kialakulásának kockázatát. Az obezitás és a hozzá kapcsolódó metabolikus diszfunkció a metabolikus szindróma előfordulásában vezető tünet. A kardiovaszkuláris halálozás előfordulása a jelenlévő rizikófaktorok számával arányosan növekszik. Úgy tűnik, hogy a túlsúly a kardiovaszkuláris funkcióra hátrányos hatással van, de a koronária mikroerek vazomotor regulációjára gyakorolt átfogó hatása még nem pontosan tisztázott. Metabolikus szindrómában a koronária mikroerek vazomotor funkciójának károsodása alakulhat ki, amely a miokardialis diszfunkció kialakulását eredményezheti.

A disszertációban az obezitás hatását vizsgáltuk a vazomotor funkcióra a nagy arteriákban, és a koronária arteriolákban. Azt találtuk, hogy az túlsúlyos betegek arteria brachialisában a flow-mediálta, és a nitroglicerín-indukálta dilatáció, és a body-mass index (BMI) között pozitív korreláció mutatható ki. Diéta indukálta obes állatmodell segítségével bebizonyítottuk, hogy a solubilis guanilát cikláz (sGC) aktivitásának fokozódása következtében a vaszkuláris simaizom sejtek érzékenysége nitrogén-oxidra megnövekszik, ami a koronária arteriolák vasodilatációjának növekedését eredményezheti. A túlsúlyos egyedek arteriái adaptálódnak a hemodinamikai változásokhoz up-regulációs sejtmechanizmusokon keresztül. A mechanizmus jobb megértése hozzájárulhat a vaszkuláris adaptációhoz, bepillantást adhat a patológiai események sorozatába obesitas esetén, és ezen hatások terápiás célra történő felhasználását segítheti. Jelenleg még nagy kihívást jelent a morbiditás és mortalitás csökkentése a metabolikus szindrómában szenvedő betegek körében.

A munkám során célul tűztük ki, megvizsgálni az obezitás hatását a humán arteria brachialis endothelium dependens, és –independens vasodilatációjára. Valamint megvizsgálni az obezitás hatását állatmodellben a koronária arteriolák vazomotor funkciójára, megvizsgálni a vaszkuláris adaptációt, és feltérképezni a folyamatban részt vevő sejtszintű mechanizmusokat.

A metabolikus szindrómában a nagyerek atherosclerosisának idő előtti kialakulása figyelhető meg. Az atherosclerosis korai fázisában az endothel diszfunkció már kimutatható, mielőtt az érfal strukturális elváltozásai kialakulnának. A koronária mikroerek működésének károsodása a szív szöveti keringésének megváltozásához vezet, ami aztán ischaemias szívkárosodás formájában nyilvánulhat meg.

Humán vizsgálatok során a nagy arteriák (arteria brachialis) endothel funkcióját a végtag 5 percig tartó leszorítása utáni felengedés hatására kialakuló érátmérő változásával vizsgáltuk, mely az áramlásfüggő endothel-dependens dilatációval áll kapcsolatban. Vizsgálatunkban az arteria brachialisban mérhető flow-mediálta vazodilatáció (FMD) és a nitroglicerín-mediálta vazodilatáció szignifikánsan magasabb volt az obes betegek csoportjában (BMI>30), mint a sovány betegek esetében (BMI<25). A BMI és az FMD, és a BMI és a nitroglicerín-indukálta dilatáció között is pozitív korrelációt találtunk.

Állatkísérletek során az elhízás szintén az endothelfüggő érválasz károsodásához vezetett. Az endothel függő dilatációt acetilkolin (ACh) segítségével vizsgáltuk kövér és sovány patkányok koronaria arterioláin. Az ACh kiváltotta érválaszokat az endotheliális NO szintézt gátló N-nitro-L-arginin-metilészter (L-NAME) 30 perces inkubációját követően is megvizsgáltuk. Ezt követően az arteriolák ACh-ra adott érválaszát szolubilis guanilát cikláz (sGC) gátló oxadiazolo-quinoxaline (ODQ) 30 perces inkubációját követően is megmértük mindkét csoportban. NO donorként a sodium nitroprusszid-ot (SNP) használtuk, és az endothel-independens dilatáció tekintetében vizsgáltuk az SNP dózisfüggő hatásait. A NO donor, SNP hatására kialakuló érválaszokat a szolubilis guanilát cikláz (sGC)-gátló oxadiazolo-quinoxaline (ODQ) 30 perces inkubációját követően is megmértük. Az sGC aktivitást meghatároztuk mind a sovány, mind az elhízott állatok koronaria arterioláiban a cGMP szint meghatározásával immunkémiai módszerekkel kiindulási állapotban, és nitrogén donor, SNP jelenlétében, valamint megmértük carotis arterián is ELISA módszert alkalmazva. Vizsgáltuk a sejtporomeabilis, cGMP-analóg 8-bromo-cGMP emelkedő koncentrációinak hatására bekövetkező érválaszt is. Western immunoblott technikával.

Eredményeinket tekintve ACh hatására fellépő, endothel-dependens dilatáció szempontjából a sovány és kövér patkányokból nyert erek válaszában nem volt szignifikáns különbség. A NO szintézis L-NAME-al történő gátlása csak a sovány állatokból nyert koronaria arteriolákban csökkentette az ACh-kiváltotta dilatációt, a kövér patkányok koronaria arterioláiban bekövetkezett dilatációra azonban ez nem volt hatással. Ebből arra lehet következtetni, hogy a kövér patkányokban a koronaria arteriolák NO szintézise károsodik. Az ACh válasz azonban azt sugallta, hogy a károsodott NO szintézis ellenére a kövér patkányok arterioláinak ACh által kiváltott dilatációja megtartott marad, aminek a hátterében eddig nem ismert kompenzatorikus mechanizmusok aktiválódása állhat. A NO donor, a natrium nitroprusszid (SNP) hatására kialakuló endothelium-independens dilatáció a kövér patkányokban szignifikánsan nagyobb volt, a kontroll csoport arterioláinak érválaszával összehasonlítva. Ez arra enged következtetni, hogy a túlsúlyos patkányok koronaria

mikroereiben az arterioláris simaizom NO érzékenysége fokozódik. A cGMP analóg 8-bromo-cGMP által kiváltott dilatáció nagysága nem különbözött a két csoport állataiból izolált erek válaszaiban, a 8-bromo-cGMP ugyanolyan mértékű dilatációt eredményezett mindkét csoportban. A fokozott NO érzékenység háttérében tehát a cGMP fokozott termelődése állhat. Ezt erősítette meg az az eredmény, miszerint a solubilis guaniláz cikláz (sGC) gátló, oxadiazolo-quinoxalin (ODQ) jelenlétében a SNP kiváltotta vazodilatáció, a sovány és túlsúlyos patkányok ereiben nem különbözött egymástól, vagyis a sGC gátlása a NO donor kiváltotta dilatációt ugyanolyan szintre csökkentette. Tehát eltűnt a két csoport között gátlószer nélkül meglévő különbség. Immunkémiai módszerekkel a kövér állatok natív koronária arterioláiban a SNP stimulálta cGMP szint szignifikánsan magasabb volt a sovány állatokhoz képest. A cGMP szintet meghatároztuk carotis arteriákban ELISA módszerrel is, melynek során az előzőhöz hasonló eredményre jutottunk. Alapállapotban a cGMP szint csaknem azonos volt a két csoportban, majd a NO-donor SNP-t hozzáadva a cGMP szint mindkét csoportban emelkedett, de a kövéreknél nagyobb mértékben. Feltételezhető, hogy a fokozott cGMP termelés háttérében a szolubilis guanilát cikláz (sGC) megnövekedett expressziója állhat, ezért megvizsgáltuk az eNOS protein expresszióját, és a sGC  $\beta$ 1 alegységének protein szintjét Western immunoblott technikával. A vizsgálat során azonban nem találtunk különbséget egyik esetben sem a két vizsgált csoport vonatkozásában. Tehát ez azt a következtetést támasztja alá, hogy a fokozott cGMP termelődést, és ennek következtében a fokozott dilatációt a solubilis guanilát cikláz aktivitásának, és nem a termelődésének a fokozódása eredményezi.

Az endoteliális NO szintézis hiányában a sGC aktivitása kompenzatórikusan fokozódik. A megnövekedett sGC aktivitás háttérében álló mechanizmusok jelenleg még nem teljesen tisztázottak. A kísérletek alapján arra lehet következtetni, hogy az elhízás eddig ismeretlen adaptációs vascularis mechanizmusokat aktivál a koronária mikroerek falában, aminek hatására a károsodott dilatáció kompenzálódása következik be. Ezáltal képes biztosítani a megfelelő myocardialis szöveti perfúziót. Elképzelhető, hogy metabolikus szindrómában a megnövekedett oxidatív stressz nem csupán az endothelium működését befolyásolja, de hatással lehet az érfal simaizom mediálta vazoregulációs mechanizmusaira is. Ezáltal fokozhatja a sGC aktivitását, ami a koronária arteriolák adaptációs folyamatait aktiválja. Mindezek alapján megállapítható, hogy az elhízás során a koronária mikroerek NO szintézise károsodik, azonban a sGC aktivitásának fokozódásával az arterioláris simaizom NO érzékenysége is fokozódik. A metabolikus szindrómában ezek a folyamatok jelentős szerepet játszhatnak a koronária mikrokeringés fenntartásában.

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**11. APPENDIX**

**I.**

**II.**

**III.**

## CITABLE ABSTRACTS

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