MECHANISMS OF VASCULAR ADAPTATION TO OBESITY

Summary of Ph.D. thesis

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PUBLICATIONS RELATED TO 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.

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.

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. 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.

Obesity and cardiovascular regulation

Complex functional and structural changes occur in the heart during the progression of obesity. It is widely accepted 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. 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. 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. It is also believed that obesity is associated with a hyperdynamic circulation and increased cardiac output. It has also been shown that total peripheral resistance inversely correlates to body mass index (BMI). 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.

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. 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; and also by the impact of several, combined risk factors present in obesity.

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

Only a limited number of studies are available that investigated alterations in vasomotor responses of coronary microvessels in obese patients. Because other studies 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. 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. 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.

Collectively, on the basis of the aforementioned clinical and experimental data 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.

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 to endothelium-dependent and –independent dilations of the human brachial artery; and
2) To investigate the impact of obesity on coronary arteriolar vasomotor function, hence to furnish evidence for vascular adaptation and to explore 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.

METHODS

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.

Ultrasound measurements of the brachial artery were performed using high-resolution ultrasound. 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. Lumen diameters were measured at least 3 times at baseline, every 20 seconds after reactive hyperemia, and subsequent to the administration of nitroglycerin. The maximum relaxations to hyperemic flow (flow mediated dilation, FMD) and to nitroglycerin (NTG) were

calcutated. Vasodilation was then calculated as the percent change in diameter over the baseline value.

Animal model of obesity

Male Wistar rats (N=50) were purchased from Charles River Laboratories. Rats were maintained on standard rat chow (N=25) or on high-fat diet (N=25), for 10 weeks. All protocols were approved by the Institutional Animal Care and Use Committee.

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, connected to a microscope.

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 μmol/L) were administered to the coronary arterioles from lean and obese rats in the presence and absence of N^{ω}-nitro-L-arginine-methyl-ester (L-NAME; 200 μmol/L, for 30 min), and changes in diameter were measured. Then, arterioles were incubated with soluble guanylate cyclase (sGC) inhibitor, oxadiazolo-quinoxaline (ODQ, 10 μmol/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 μmol/L) were investigated in isolated coronary arterioles of lean and obese rats. Then, arterioles were incubated with ODQ (10 μmol/L, for 30 min) and arteriolar responses to NO donor was reassessed. In another series of experiments increasing concentrations of 8-bromo-cGMP (1 nmol/L – 10 μmol/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^{2+}-free medium.

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. Briefly, the left ventricle including the coronary arteriole was embedded and frozen in optimal cutting temperature compound. Unfixed consecutive sections (10-μm thick) were transferred to a solution containing 1 μM SNP in physiological salt solution (PBS) and incubated for 15 min at 37 ºC. Other sections remained in PBS solution during the 15-min incubation period and served as unstimulated controls. Sections were than fixed with acetone and immunolabeled with a monoclonal anti-cGMP primary antibody. Immunostainings were visualized by using avidin-biotin horseradish peroxidase visualization system, stained with diaminobenzidine (DAB). Images of the sections were collected with a digital camera connected to a microscope. For semi quantitative analysis of the cGMP immunoreactivity, in defined areas of the arteriolar wall the amount of the brown product (DAB) was estimated by measuring optical density. Background subtracted, averaged optical density was then calculated and compared in coronary arterioles of lean and obese rats.

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 following the instructions by the manufacturer.

Immunoblots

Single coronary arteries were dissected from the hearts cleared of connective tissue and briefly rinsed in ice-cold PBS. After the addition of 20 μl of Laemmli sample buffer tissues were homogenized. The antibodies used for detection of protein expression (anti-eNOS IgG and anti-sGC ß1 subunit IgG) were obtained from Sigma Inc. Anti-ß-actin IgG was used as loading control. Signals were revealed with chemiluminescence and visualized autoradiographically. Optical density of bands was quantified and normalized for ß-actin.

Statistical analysis

Data were stored and analyzed with the NCSS statistical software. One-way ANOVA followed by Tukey posthoc test was performed to compare differences in brachial artery responses. 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 by two-way repeated-measures ANOVA followed by Tukey's post hoc test or Student's t-test as appropriate. Data are expressed as means±S.E.M. $P<0.05$ was considered statistically significant.

RESULTS

Brachial artery dilations to hyperemic flow and nitroglycerin in patients

In order to reveal the impact of obesity on vasomotor function brachial artery dilations were assessed by high resolution ultrasound. 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±0.6%. 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 were significantly enhanced in the obese vs. lean (or overweight) subjects (FMD, obese vs. lean: $P=0.026$, obese vs. overweight: $P=0.011$ and NTG, obese vs. lean: $P=0.023$). Correspondingly, Pearson correlation revealed a significant, positive correlation between BMI and FMD and also between BMI and NTG-induced 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.

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: $P=0.611$, β-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, β-blockers: *P*=0.619, ACE-inhibitors: *P*=0.980, Diuretics: *P*=0.566, Lipid lowering drugs: p=0.570).

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. Also, we have found that the C-reactive protein levels were similar in the two groups of animals. 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 (~50%) elevated in obese rats, as compared to other animal models of type 2 diabetes, such as the db/db mice or the diabetic Zucker rats, in which animals have about 4-times higher fasting glucose levels.

In coronary arterioles isolated from lean and obese rats there were no significant differences between the spontaneously developed arteriolar tone (86±6 μm and 97±6 μm, respectively) and in the passive arteriolar diameters (in Ca^{2+}-free medium, 153±9 μm and 149±7 μm, 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. Inhibition of NO synthesis with L-NAME decreased ACh-induced dilation in coronary arterioles isolated from lean animals, whereas it had no significant effect on ACh-induced responses in arterioles of obese rats. 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.

Dilations to the NO donor, sodium nitroprusside (SNP) was 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. SNP-induced dilations were also obtained after administration of ODQ, an inhibitor of soluble guanylate cyclase. ODQ decreased SNP-induced dilations, thereby eliminating the NO-donor evoked differences in dilations between the two groups. In separate series of experiments arteriolar responses were obtained to 8-bromocGMP, a cell permeable, stabile 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.

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.

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. 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.

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 and also in the soluble guanylate cyclase ß1 subunit protein levels in coronary arterioles of lean and obese rats.

DISCUSSION

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 dependent on the responsiveness of vascular smooth muscle to NO. 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. A study has reported that in patients with morbid obesity, rapid weight reduction is associated with reduction of NO synthesis (Atherosclerosis 190: 436-442, 2007). 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. In this set of experiments we have found that in coronary arterioles (active diameter less than 100 μm) 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, 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. Given that, in obesity coronary resistance vessels should adopt 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. 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 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. It has been posited that an enhanced sensitivity of the downstream sGC to NO may compensate for the reduced NO availability. In this context, it has been also found that acute administration of exogenous NO decreased sGC activity and in long-term its protein expression. These findings indicate that NO may play an important, negative feed back 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 donors (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. Furthermore, our cytochemistry data suggested 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 β1 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. It has been shown that TNFα-mediated vascular inflammation have important consequences with respect to downstream signaling of NO. 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.

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. 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. 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 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.

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