

Coronary Endothelial Dysfunction, Obesity and Metabolic Syndrome

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Abstract

Objective To define the impact of metabolic syndrome (MetS) and obesity on coronary vascular function, with the hypothesis that subjects with MetS will have endothelial dysfunction.

Background: Obesity or the metabolic syndrome (MetS) is associated with a higher risk of diabetes and coronary artery disease (CAD). Endothelial dysfunction is a common causal pathway in the initiation and progression of CAD.

Methods: A total of 418 patients (165 obese, 239 MetS) with and without angiographic evidence of CAD underwent coronary vascular function testing by measuring coronary blood flow (CBF) velocity in response to intracoronary infusion of acetylcholine (ACH) and sodium nitroprusside (SNP) and coronary flow reserve with adenosine.

Results: Endothelium-dependent microvascular vasodilation correlated with BMI ($r=-0.12$, $p=0.02$), with ACH responses significantly lower in overweight, obese and MetS subjects ($p=0.003$). The number of MetS components correlated with the response to ACH in both the coronary microcirculation and the epicardial coronary arteries, and with impaired coronary microcirculatory responses to adenosine. No significant correlation was observed with SNP. In multivariable analysis, beyond age, only the total number of MetS components, and not BMI, emerged as an independent predictor of impaired microvascular response to ACH (CBF: $\beta=-0.18$, $P<0.001$). Low-grade inflammation (C-reactive protein) was higher in patients with MetS, but was not associated with coronary vascular function.

Conclusions: We demonstrate that the clustering of MetS components is an important and independent determinant of coronary endothelial dysfunction in subjects with and without CAD.

Keywords: Atherosclerosis; Metabolic syndrome; Obesity; Endothelium; Inflammation

Abbreviations: ACH: Acetylcholine; CAD: Coronary Artery Disease; CBF: Coronary Blood Flow; CVD: Cardiovascular Disease; CVR: Coronary Vascular Resistance; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein; MetS: Metabolic Syndrome; NCA: Normal Coronary Artery; SNP: Sodium Nitroprusside

Introduction

The prevalence of obesity has increased dramatically worldwide whereby at least a third of adult Americans are obese and two-third overweight [1,2]. Obese subjects are at high risk of multiple morbidities, foremost among which are the development of diabetes and cardiovascular disease (CVD) [3,4]. Abdominal obesity in particular is frequently associated with a clustering of multiple interrelated cardiovascular risk factors associated with insulin resistance that characterizes the metabolic syndrome (MetS). In addition to a large waist circumference, subjects with MetS have increased blood pressure, dyslipidemia characterized by low HDL, high triglycerides and small dense LDL particles, and glucose intolerance commonly accompanied by low-grade systemic inflammation and a pro-thrombotic state [3,5]. The clustering of three or more of these risk factors in an individual defines the MetS [6], the incidence of which has progressively risen [2,7,8]. Because of its frequent association with the MetS variables, the role of obesity as an independent risk factor for CVD remains controversial [2,9-11].

Endothelial dysfunction, often a consequence of exposure of the vasculature to risk factors, is predictive of adverse cardiovascular outcomes, and appears to provide a common causal pathway in the initiation and progression of CVD [12-15]. Peripheral arterial endothelial dysfunction has been observed in obese children and

adults, and in those with MetS, even after adjustment for conventional risk factors [16-21]. Although an independent association between obesity and coronary endothelial dysfunction has been described [22], the incremental influence of the MetS variables and low-grade systemic inflammation on coronary endothelial function remains unknown. Herein we investigated these relationships in a large, well-characterized cohort of patients with and without angiographic evidence of coronary artery disease (CAD) undergoing invasive assessment of coronary vascular function. Our hypothesis was that MetS will be associated with more profound and selective dysfunction of the coronary vascular endothelium.

Methods

Patients

We prospectively studied 418 patients (239 males) undergoing diagnostic cardiac catheterization for evaluation of symptoms of chest pain or abnormal cardiac stress test findings. CAD was defined as angiographic evidence of plaque or more severe occlusive disease, and

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normal coronary arteries (NCA) defined as angiographically smooth appearing coronary arteries. Patients with 3-vessel disease, recent myocardial infarction, severe heart failure or valvular heart disease were excluded. Diabetes was defined as a fasting blood glucose level ≥ 126 mg/dL or treatment with dietary modification, insulin, or oral hypoglycemic agents at the time of the study. Hypercholesterolemia was defined as a fasting serum total cholesterol >240 mg/dL or if the subject was being treated with lipid-lowering medication or dietary modification. Hypertension was defined as a seated systolic blood pressure >140 mm Hg or diastolic pressure >90 mm Hg on at least 3 occasions, or if such a diagnosis had been made in the past and the patient was being treated with medications or lifestyle modification.

Cardiac medications were withdrawn for ≥ 48 hours and at least 5 half-lives before the study. Angiotensins converting enzyme inhibitors and aspirin or other cyclooxygenase inhibitors were discontinued ≥ 7 days before the study. The protocol was approved by the Institutional Review Board of the National Heart, Lung and Blood Institute, and informed written consent was obtained from each subject.

Anthropometric variables and atherosclerosis risk factors

Subjects had their height (m) and weight (kg) measured and body mass index (BMI) calculated ($\text{weight}/\text{height}^2$). Subjects were categorized as normal weight if BMI was <25 kg/m^2 , overweight if BMI was between 25 and <30 kg/m^2 , and obese if BMI was ≥ 30 kg/m^2 . Blood pressure, fasting lipid profile, fasting glucose and high sensitivity C-reactive protein (CRP) levels were also measured. All subjects with a history of current or prior tobacco smoking were classified as smokers.

Subjects were also categorized according to a modified definition of the NCEP/ATPIII criteria for MetS. Waist circumference data were not available for most subjects; therefore BMI values were used to define adiposity. Five MetS components were identified: 1 - Overweight or obesity (BMI ≥ 25 kg/m^2); 2 - elevated blood pressure ($\geq 130/\geq 85$ mmHg or treatment with antihypertensive medication); 3 - elevated triglycerides (≥ 150 mg/dL); 4 - low HDL (<40 mg/dL [male] or <50 mg/dL [female]); 5 - elevated fasting glucose (≥ 110 mg/dL or treatment with glucose lowering medication). Subjects were deemed to have MetS if they fulfilled 3 or more of these criteria.

Vascular function studies

A 6-French guide catheter was introduced into an unobstructed ($<30\%$ stenosis) coronary artery, and coronary blood flow (CBF) velocity was measured using a 0.014- or 0.018-inch Doppler flow wire (Flowire, Volcano Corp, Rancho Cordova, CA) as described previously [23]. Endothelium-dependent vasodilation was estimated by measuring CBF responses to an infusion of intracoronary acetylcholine (ACH) at a rate of 15 $\mu\text{g}/\text{min}$ for 2 minutes to obtain an estimated 10^{-6} mol/L intracoronary concentration. Endothelium-independent vasomotion was estimated with intracoronary sodium nitroprusside (SNP) (20 $\mu\text{g}/\text{min}$) infusion for 3 minutes. When drugs were infused into the left main coronary artery, the infusion rate was doubled. Coronary flow reserve was determined with adenosine infused at 2.2 mg/min for 2 minutes.

For calculating CBF, diameter was measured in a 0.25 to 0.5 cm segment of vessel beginning 0.25 cm beyond the tip of the flow wire. CBF was determined from the Doppler-derived flow velocity and diameter measurements using the formula: $\pi \times \text{average peak velocity} \times 0.125 \times \text{diameter}^2$ as previously described [23]. Coronary vascular resistance (CVR) was calculated as mean arterial pressure divided by CBF. Additionally, mid and distal segments of the study vessel that

were straight and free of overlap or major branch points were also measured after each intervention. Epicardial coronary responses in these segments were determined by assessment of the percent change in diameter (ΔDiam) with each drug compared to baseline. Quantitative angiography was performed with the ARTREK software (Quantim 2001, Statview, Image Comm Systems, Inc) or PIE medical CAAS system [24].

Statistical analysis

Continuous variables are expressed as mean value \pm standard deviation (SD). Normality was tested using the Kolmogorov-Smirnov criterion. Logarithmic transformation was performed for skewed distributions before any parametric analyses. Skewed variables are expressed as a median value (interquartile range). Categorical data are expressed as absolute frequencies and percentages. Comparisons between 2 groups were performed using the student's t-test for unpaired measures (continuous data) and Pearson's chi-square test (categorical data). Univariate correlations were performed using the Pearson's correlation coefficient. Linear trends between the number of MetS components and coronary function indices were evaluated by a one way analysis of variance. Multivariable analysis adjusting for potential confounders was performed by either analysis of co-variance (ANCOVA) or forward linear regression analysis. The assumptions for linearity and homoscedasticity were tested based on the standardized residuals plots. Exact P values < 0.05 were considered statistically significant. Data analysis was performed with SPSS software, version 14.0 (SPSS Inc, Chicago, IL).

Results

Subject characteristics

Of the 418 patients (239 males and 179 females) enrolled, 165 were obese, 163 overweight, and 239 (57.2%) fulfilled the criteria for MetS. Clinical characteristics of the population according to the presence of obesity and MetS are shown in Table 1. As expected, the presence of MetS was associated with a higher frequency and severity of all of the components of MetS in addition to diabetes. Overweight/obese patients also had a higher incidence of these risk factors, except for diabetes. Total and LDL cholesterol levels were similar in the subgroups. Overall, patients with MetS had a higher prevalence of CAD.

Individual risk factors and vascular responses

Microvascular responses: Endothelium-dependent microvascular vasodilation, measured as the % increase in flow with ACH, correlated with BMI ($r=-0.12$, $p=0.02$). The ACH responses were significantly lower in both overweight and obese compared to normal weight subjects (100% in overweight/obese vs. 135% in subjects with normal weight, $p=0.003$) Figure 1. Responses to ACH were similar in the overweight and obese subjects. The % increase in CBF with ACH also correlated with age ($r=-0.17$, $p=0.001$) and HDL levels ($r=0.12$, $p=0.021$) and was diminished in those with elevated blood pressure (95% in hypertensives vs. 122% in normotensives, $p=0.014$), diabetes (70% in diabetics vs. 117% in non-diabetics, $p=0.001$) and CAD (99% in CAD patients vs. 117% in patients without CAD, $p=0.02$). Following multivariable adjustment (ANCOVA), overweight/obesity remained an independent predictor of impaired flow response to ACH ($p=0.049$) along with age ($p=0.003$) and diabetes ($p=0.013$). Similar relationships were observed between abnormal BMI and the change in CVR in response to ACH after both univariable (Figure 1) and multivariable analysis. Furthermore, if BMI was considered as a continuous variable in the multivariable model, it remained a significant predictor of

	All patients	Normal weight	Overweight/obese	P-value	No MetS	MetS	P-value
N (%)	418	90 (22)	328 (78)		179 (43)	239 (57)	
Age, years	55.2 ± 11.3	55.8 ± 13.0	55.1 ± 10.8	0.59	53.7 ± 10.3	56.3 ± 10.3	0.021
Gender, males/females	239/179	49/41	190/138	0.55	91/88	148/91	0.023
Weight, kg	85 ± 19	66 ± 9	90 ± 18	<0.001	77 ± 17	91 ± 19	<0.001
Height, cm	169 ± 10	168 ± 10	169 ± 11	0.64	168 ± 10	169 ± 11	0.2
BMI, kg/m ²	29.7 ± 6.2	22.7 ± 2.1	31.6 ± 5.5	<0.001	27.1 ± 5.8	31.7 ± 5.8	<0.001
Mean blood pressure, mmHg	109 ± 15	105 ± 15	110 ± 15	0.018	105 ± 15	111 ± 15	<0.001
Total cholesterol, mg/dL	214 ± 46	209 ± 48	216 ± 46	0.21	212 ± 48	216 ± 45	0.34
LDL-cholesterol, mg/dL	138 ± 42	132 ± 45	140 ± 41	0.13	139 ± 42	138 ± 42	0.77
HDL-cholesterol, mg/dL	43 ± 14	49 ± 17	42 ± 12	<0.001	51 ± 15	38 ± 9	<0.001
Triglycerides, mg/dL	145(97–218)	112(70–165)	151(106–229)	0.02	108(76–136)	184(135–261)	<0.001
Glucose, mg/dL	101(92–120)	96(89 107)	103(93–126)	0.002	95(88–102)	112(98–140)	<0.001
hsCRP, mg/L	0.82(0.46–1.15)	0.59(0.40–0.89)	0.91(0.48–1.20)	0.043	0.59(0.25–0.93)	0.99(0.65–1.34)	0.005
Risk factors							
Hypertension, N (%)	213 (51)	35 (39)	178 (55)	0.13	47 (26)	166 (70)	<0.001
Diabetes, N (%)	89 (22)	15 (4)	74 (18)	0.004	7 (4)	74 (31)	<0.001
Hypercholesterolemia, N (%)	261 (62)	52 (58)	209 (64)	0.16	103 (62)	158 (75)	0.007
Smoking, N (%)	245 (59)	54 (60)	191 (59)	0.83	96 (54)	149 (63)	0.051
CAD, N (%)	215 (51)	40 (44)	175 (53)	0.13	69 (39)	146 (61)	<0.001
Diseased coronary arteries	1.7±0.9	1.8±1.0	1.8±0.9	0.98*	1.6±0.9	1.8±0.9	0.10*

BMI: Indicates Body-Mass Index; CAD: Coronary Artery Disease; hsCRP: High-Sensitivity C-Reactive Protein, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein. Categorical variables are presented as absolute (relative) frequencies; continuous variables, as mean ± SD or median (interquartile range).

P-values for between groups comparisons are derived from student's t-test for unpaired measures (for continuous variables) or from chi-square tests (for categorical variables).

*P-value for comparison within the CAD patients.

Table 1: Baseline characteristics of the whole study population and according to presence of overweight/obesity and metabolic syndrome (MetS).

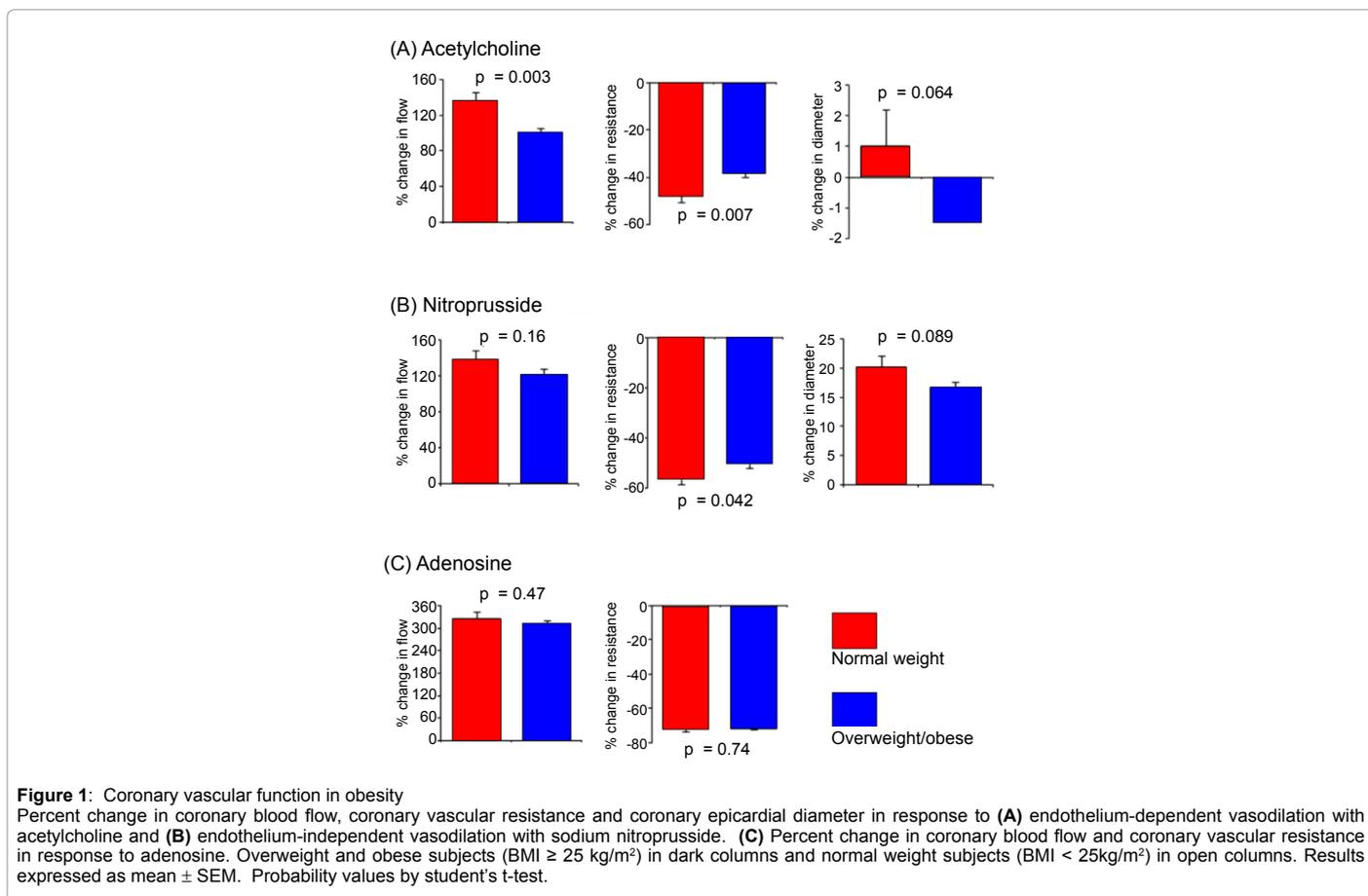


Figure 1: Coronary vascular function in obesity

Percent change in coronary blood flow, coronary vascular resistance and coronary epicardial diameter in response to (A) endothelium-dependent vasodilation with acetylcholine and (B) endothelium-independent vasodilation with sodium nitroprusside. (C) Percent change in coronary blood flow and coronary vascular resistance in response to adenosine. Overweight and obese subjects (BMI ≥ 25 kg/m²) in dark columns and normal weight subjects (BMI < 25kg/m²) in open columns. Results expressed as mean ± SEM. Probability values by student's t-test.

impaired ACH responses (CBF: standardized $\beta = -0.13$, $p = 0.014$; CVR: $\beta = -0.1$, $p = 0.05$).

In contrast, BMI was not correlated with the flow response to the endothelium-independent vasodilator, SNP, or the flow and resistance responses to adenosine (Figure 1). Although overweight/obesity was related to a lower resistance response to SNP (Figure 1), this was no longer significant after adjustment for aforementioned covariates.

Epicardial responses: Age ($r = -0.10$, $p = 0.048$) and triglyceride levels ($r = -0.11$, $p = 0.028$) correlated with epicardial responses to ACH, as was presence of CAD (mean % diameter change of -2.3% in CAD vs. 0.5% in no CAD patients, $p = 0.002$). A trend towards epicardial vasoconstriction with ACH was observed in overweight/obese subjects compared to those with normal BMI, and a weaker trend to impaired vasodilation in response to SNP was also observed (Figure 1). Following multivariate adjustment, there was no independent association between the presence of overweight or obesity and an impaired dilator response to ACH or SNP (both $p = NS$).

Components of MetS and coronary vascular function

The impact on vascular function of other components of the MetS in addition to BMI was also studied. Although there were no significant differences in baseline measurements between subjects with or without the MetS, microvascular vasodilator responses to ACH were significantly impaired in subjects with MetS compared to those without MetS (Table 2). However, the epicardial diameter changes with ACH and endothelium-independent responses to SNP were similar in the epicardial vessels and the microvasculature. Microvascular responses to adenosine were also impaired in patients with MetS (Table 2).

In the entire population, significant correlations between the number of MetS components and the response to ACH in both the coronary microcirculation and the epicardial coronary arteries were observed (Figure 2). Differences in the epicardial circulation became apparent between individuals with none or one component of MetS and those with 2 or more components ($P = 0.035$). Furthermore, a linear trend between the number of components of MetS and impaired coronary microcirculatory responses to adenosine was observed

(Figure 2). No significant correlation was observed between the number of MetS components and responses to SNP. Thus, exposure to increasing number of risk factors of MetS was associated with greater endothelial dysfunction in both the epicardial coronaries and coronary microcirculation, and with diminished coronary flow reserve.

To investigate further the impact of individual components of the MetS on coronary vascular responses to ACH, we performed multivariable forward linear regression analysis in which the individual risk factors for MetS, total number of the components of MetS (0-5), and the presence of CAD (0/1) were also introduced as covariates. Beyond age, only the total number of MetS components emerged as an independent predictor of impaired microvascular response to ACH (CBF: $\beta = -0.18$, $P < 0.001$; CVR: $\beta = -0.16$, $P = 0.002$). Thus, it is the clustering of the components of MetS rather than any individual component that best predicts abnormal coronary endothelial function in the microcirculation. In contrast, presence of CAD was the only determinant of an abnormal epicardial endothelial response to ACH ($\beta = -0.12$, $P = 0.017$) and of microcirculatory response to adenosine (CBF: $\beta = -0.33$, $P < 0.001$; CVR: $\beta = -0.30$, $P < 0.001$).

MetS, coronary vascular function and low-grade inflammation

Inflammation, estimated as Hs-CRP level, was higher in patients with MetS (Table 1). In contrast, Hs-CRP level was not associated with coronary microvascular or epicardial endothelium-dependent responses to ACH (CBF: $r = -0.015$, $P = 0.83$; CVR: $r = 0.022$, $P = 0.74$; epicardial diameter: $r = -0.022$, $P = 0.73$). In subsequent analyses where it was included as an additional covariate in the multiple regression models, Hs-CRP level did not significantly alter the relationship observed between coronary vascular function and MetS, number of MetS components, or BMI.

Discussion

We have demonstrated for the first time an independent and graded relationship between the MetS risk factor burden and coronary endothelial dysfunction. In one of the largest cohorts to date evaluating coronary vascular function, we found that MetS is associated with coronary microvascular endothelial dysfunction in those with and

	All patients	MetS	No MetS	P-value
Baseline coronary function				
CBF, mL/min	43.9 ± 31.7	45.4 ± 27.9	42.0 ± 36.0	0.29
CVR, mmHg×min/mL	3.59 ± 2.39	3.46 ± 2.37	3.77 ± 2.42	0.20
Coronary artery diameter, mm	2.62 ± 0.73	2.65 ± 0.73	2.57 ± 0.72	0.25
Responses to acetylcholine				
Change of CBF, %	108 ± 98	97 ± 93	122 ± 102	0.015
Change of CVR, %	-40 ± 29	-37 ± 31	-45 ± 27	0.008
Change of coronary diameter, %	-0.92 ± 11.01	-1.72 ± 11.32	0.12 ± 10.56	0.10
Responses to nitroprusside				
Change of CBF, %	126 ± 85	126 ± 81	125 ± 90	0.95
Change of CVR, %	-52 ± 21	-52 ± 19	-51 ± 23	0.50
Change of coronary diameter, %	17.40 ± 14.07	16.85 ± 14.38	18.16 ± 13.64	0.45
Responses to adenosine				
Change of CBF, %	314 ± 157	298 ± 156	335 ± 157	0.03
Change of CVR, %	-72 ± 11	-71 ± 11	-74 ± 10	0.029

BMI: Body-Mass Index; CAD: Coronary Artery Disease; CBF: Coronary Blood Flow; CVR: Coronary Vascular Resistance

Categorical variables are presented as absolute (relative) frequencies; continuous variables, as mean ±SD.

P-values for comparisons between patients with and without MetS are derived from student's t-test for unpaired measures.

Table 2: Coronary artery characteristics of the whole study population and according to presence of metabolic syndrome (MetS).

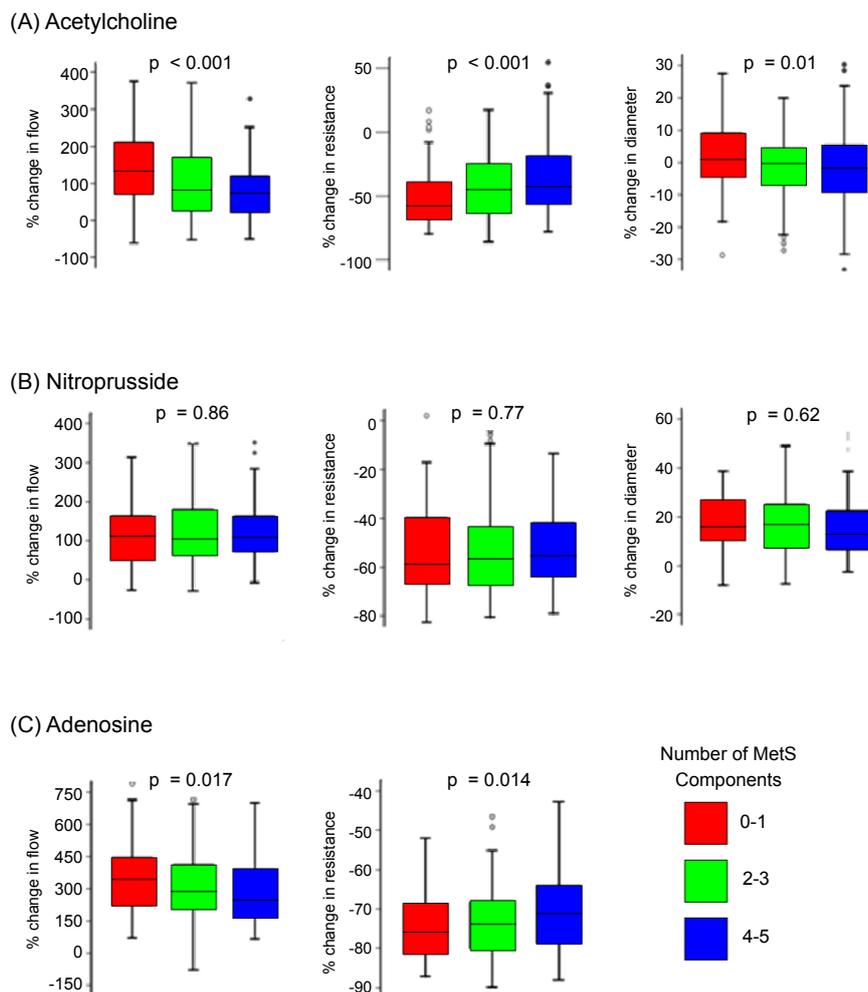


Figure 2: Coronary vascular function and metabolic syndrome

Percent change in coronary blood flow, coronary vascular resistance and coronary epicardial diameter in response to (A) endothelium-dependent vasodilation with acetylcholine and (B) endothelium-independent vasodilation with sodium nitroprusside. (C) Percent change in coronary blood flow and coronary vascular resistance in response to adenosine. Subjects with 0/1 component of the MetS in open boxes, 2/3 components of the MetS in speckled boxes and 4/5 components of the MetS in grey boxes. The center line of the box denotes the median value; the extremes of the box, the inter-quartile range; and the bars, the upper and lower limits of 95% of the data. Probability values by 1-way ANOVA.

without atherosclerosis. In particular, a striking relationship emerged between increasing MetS risk factor burden and coronary endothelial dysfunction; for every additional component of MetS, the coronary flow response to ACH was approximately 13% lower. Although obesity appeared to be an independent predictor of coronary microvascular responses, this relationship was no longer apparent when the number of MetS components was introduced into the model, suggesting that it is the burden of metabolic risk factors in the context of obesity which are most likely to determine the coronary pathology rather than the body habitus per se. Notably, neither obesity nor MetS (whether considered as a discrete entity or as number of MetS components) was related to endothelium-independent coronary vascular responses, indicating that the abnormal response to ACH is most likely due to an abnormality in the endothelial layer rather than the ability of the coronary smooth muscle to respond to exogenous nitric oxide. Intriguingly, we also found that the microvascular flow reserve in response to intracoronary infusion of adenosine was depressed in subjects with MetS.

The endothelium is a fundamental regulator of vascular

homeostasis. Alteration in endothelial function is not only one of the earliest recognizable changes in the atherosclerotic disease process, but is also an indicator of an increased risk of later clinical complications [13,14,24-27]. Exposure to conventional risk factors for atherosclerosis results in endothelial dysfunction which is, in part, explained by the risk factor burden [28-30] and is predictive of future cardiovascular events even in vascular territories remote from the site of testing [14,31,32]. In fact, recent studies have shown that endothelial dysfunction predates future development of hypertension and diabetes, and predicts more rapid progression of atherosclerosis [33,34]. While non-invasive methods assessing peripheral vascular endothelial function appear to correlate modestly with coronary endothelial status, and clearly have great value for assessment of low to intermediate risk groups, invasive testing remains the “gold-standard” technique for assessment of coronary vascular physiology [35,36].

The MetS is a clustering of the risk factors characterized by abdominal adiposity, hypertension, dyslipidemia (low HDL, high triglycerides and small dense LDL particles) and impaired glucose

homeostasis characteristic of insulin resistance [7]. Although under normal conditions insulin promotes the release of NO from normal endothelium, when individuals have developed tissue insensitivity to insulin, there is clear evidence of reduced endothelial NO availability [37-39]. We found a statistically independent association between MetS and coronary microvascular endothelial dysfunction particularly in relation to increasing MetS risk factor burden. Our results are in agreement with findings in the peripheral circulation where endothelial responses to pressure alterations to finger cuff (using digital arterial tonometry), to increased flow in the brachial artery and to ACH in the femoral microcirculation were impaired in subjects with MetS [16,19,20]. Although we also observed increasing epicardial endothelial dysfunction with exposure to MetS components, this relationship did not persist after adjustment for the presence of CAD.

The lower microcirculatory responses to both ACH and adenosine suggest that both endothelial function and flow reserve, respectively, are adversely affected by MetS. However, the magnitude of reduction in flow reserve (10%) in the presence of MetS was far more modest than the nearly 25% reduction in flow response to ACH, indicating a proportionately greater effect on the endothelium. Vasodilation in response to adenosine is multifactorial in nature, including a small but significant contribution of nitric oxide (NO) [40]. Thus adenosine responses, unlike the response to SNP, cannot be considered to entirely represent endothelium-independent function. It is therefore possible that the reduced response we observed with adenosine in subjects with MetS may also reflect reduced availability of NO. Nevertheless the reduced coronary flow reserve may contribute to reduced vasodilation during physiologic stress such as exercise in these patients, potentially contributing to myocardial ischemia.

Mechanisms responsible for the development of endothelial dysfunction in obesity include increased levels of oxidative stress leading to reduced NO bioavailability [41], reduced generation of endothelium-derived hyperpolarizing factor [42] and increased production of endothelium-dependent constricting factors such as endothelin-1 [43,44]. Of the individual components of MetS, the presence of obesity was a consistent determinant of vascular endothelial dysfunction in the coronary microcirculation in our study. Several investigators have previously shown both in adults and children that obesity is independently associated with impaired peripheral endothelial function and that this can be improved by appropriate lifestyle interventions [17,18,38,41,45-47]. One previous study examining coronary microvascular endothelial function confirmed these finding in a population with minimal or no coronary artery disease [22]. Our study extends these observations to patients with CAD and in the context of the associated MetS risk factor burden.

The cluster of abnormalities that emerge with visceral obesity may also have both direct and indirect adverse effects on the vascular endothelium. When the total number of components of MetS was introduced as a covariate, obesity was no longer a predictor of endothelial dysfunction in our study. Thus, it appears to be the clustering of the components of MetS that typically associate with increasing obesity, that best predict an increased risk of endothelial dysfunction rather than the obesity itself or any one of the other associated individual risk factors.

Visceral adipose tissue is a rich source of pro-inflammatory cytokines such as TNF- α and IL-6 which also contribute to both insulin resistance and endothelial dysfunction [48]. Moreover, markers of inflammation, such as elevated CRP levels, and endothelial dysfunction have both been associated with poor long term prognosis in subjects

with and without known CAD [25,49-51]. Although a previous study found a correlation between CRP levels and forearm vascular endothelial function, adjustment for obesity or MetS was not performed [52]. Others have confirmed our observations [16,22,53]. Furthermore, despite higher CRP levels in obese subjects and in those with MetS as previously reported, the association between endothelial dysfunction and MetS was not affected by further adjustment for CRP levels. These observations suggest that the degree of coronary endothelial dysfunction is best explained by the conventional MetS risk factor burden rather than the level of systemic inflammation as assessed by CRP levels. Indeed, during long term follow-up of a subset of these subjects, we found that adverse cardiovascular events were predicted by presence of coronary endothelial dysfunction and not by CRP levels [14].

Limitations

Although BMI is a good marker for increased risk of adverse "cardiometabolic" outcomes, the subjects in this study were not categorized according to abdominal circumference or measures of body fat content and distribution which are most closely linked to an adverse risk state. Additionally, data on other measures of body fat distribution such as dual-energy x-ray absorptiometry (DEXA) scan was not available for these subjects. However, viscerally obese subjects with a normal BMI are rare when compared to those with an elevated BMI [21,54]. Although we observed a correlation between obesity or MetS and the epicardial responses to ACH after univariate analysis, these differences were no longer significant after adjustment for other risk factors. Because coronary atherosclerosis causes epicardial constriction with ACH, and the majority of our cohort had CAD, we may have underestimated the influence of these factors on epicardial endothelial function in this cohort.

Since this is a cross sectional study, its findings do not infer causality between obesity or MetS and endothelial dysfunction. However, the emergence of obesity as an independent risk factor in recent surveys linking it to cardiovascular events supports our observations [55]. Further, although the ability of angiography to confirm a diagnosis of normal coronaries is limited, as eccentric atheroma is often undetectable with this technique, those with abnormal angiographic appearances are likely to have a greater disease burden than those with angiographically "smooth" vessels. Finally, our cohort consists of highly selected patients and although not entirely representative of the population as a whole, is well representative of subjects with and at risk of CAD undergoing cardiac catheterization in routine clinical practice.

Clinical Implication

We have shown that clustering of MetS components is an important and independent determinant of coronary endothelial dysfunction in subjects with and without CAD. Since, endothelial dysfunction predates development of overt disease, aggressive risk factor prevention and earlier therapeutic interventions to ameliorate endothelial dysfunction in these individuals are likely to be of great value and require further study.

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