Altered Mechanism of Adenosine-Induced Coronary Arteriolar Dilation in Early-Stage Metabolic Syndrome

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Onset of the combined metabolic syndrome (MetS) is a complex progressive process involving numerous cardiovascular risk factors. Although patients with established MetS exhibit reduced coronary flow reserve and individual components of the MetS reduce microvascular vasodilation, little is known concerning the impact of early-stage MetS on the mechanisms of coronary flow control. Therefore, we tested the hypothesis that coronary arteriolar dilation to adenosine is attenuated in early-stage MetS by reduced A_2 receptor function and diminished K^+ channel involvement. Pigs were fed control or high-fat/cholesterol diet for 9 weeks to induce early-stage MetS. Coronary atheroma was determined *in vivo* with intravascular ultrasound. *In vivo* coronary dilation was determined by intracoronary adenosine infusion. Further, apical coronary arterioles were isolated, cannulated and pressurized to 60 cmH_2O for *in vitro* pharmacologic assessment of adenosine dilation. Coronary atheroma was not different between groups, indicating early-stage MetS. Coronary arteriolar dilation to adenosine (*in vivo*) and 2-chloroadenosine (2-CAD; *in vitro*) was similar between groups. In control arterioles, 2-CAD-mediated dilation was reduced only by selective A_{2A} receptor inhibition, whereas only dual $A_{2A/2B}$ inhibition reduced this response in MetS arterioles. Arteriolar A_{2B}, but not A_{2A}, receptor protein expression was reduced by MetS. Blockade of voltage-dependent K^+ (K_v) channels reduced arteriolar sensitivity to 2-CAD in both groups, whereas ATP-sensitive K^+ (K_{ATP}) channel inhibition reduced sensitivity only in control arterioles. Our data indicate that the mechanisms mediating coronary arteriolar dilation to adenosine are altered in early-stage MetS prior to overt decrements in coronary vasodilator reserve. Exp Biol Med 234:683–692, 2009

Key words: Ossabaw miniature swine; diabetes; coronary blood flow; potassium channel; obesity; adenosine receptor

Introduction

The development of metabolic syndrome (MetS) and the subsequent onset of complications are progressive multifactorial processes. Due to the clustering of cardiovascular risk factors (insulin resistance, glucose intolerance, dyslipidemia, hypertension and obesity), patients with MetS have an increased risk of death from cardiovascular disease, particularly coronary vascular disease (1). In addition, MetS is associated with defects in the microvascular mechanisms of coronary blood flow control (2). The nucleoside adenosine plays an important role in the maintenance of coronary blood flow, particularly during episodes of cardiac ischemia (3, 4). Recently, it was demonstrated that patients with established MetS exhibit reduced coronary dilation in response to intravenous infusion of the adenosine uptake inhibitor dipyridamole (5). It is important to note, however, that these measures are collected at clinically relevant endpoints following the establishment of disease and therefore may have limited utility in determining the mechanistic changes leading to dysfunction in the early stages of disease progression. Whether or not coronary microvascular reactivity to adenosine is altered in the early stages of MetS is not known.

In the coronary microvasculature, vasodilation to adenosine is mediated primarily through the activation of adenosine type 2A (A_{2A}) and 2B (A_{2B}) receptors on vascular smooth muscle (6–8). Downstream signaling via cAMP-dependent and -independent mechanisms induces smooth muscle hyperpolarization and relaxation particularly

This work was supported by National Institutes of Health Grants HL-52490, AR-048523, RR-013223, and HL-062552, Purina TestDiet, Inc. (Richmond, IN), the Purdue-IUSM Comparative Medicine Program and American Heart Association Predoctoral Fellowships (LB, XL).

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Received December 5, 2008.
Accepted February 27, 2009.

DOI: 10.3181/0812-RM-350
1535-3702/09/2346-0683$15.00

through opening of K^+ channels (6, 9–11). To date, the impact of MetS (at any time point) on the role of A_2 receptors in coronary arteriolar dilation to adenosine has not been examined. Previous studies, however, have demonstrated that components of the MetS significantly impact coronary microvascular smooth muscle K^+ channel function (12–15). Specifically, diabetic dyslipidemia reduced steady-state K^+ current (12), hypercholesterolemia attenuated the function of smooth muscle voltage-dependent K^+ (K_v) channels (13) and diabetes impaired function of K_v and ATP-sensitive K^+ (K_{ATP}) channels in coronary arterioles (14, 15). Acute hyperglycemia was reported to reduce arteriolar K_v channel function in isolated rat coronary arteries (16). Thus, in the combined disorder of the MetS, these risk factors may lead to differential alterations in the receptors and/or ion channels mediating coronary reactivity to adenosine. Therefore, we sought to determine the impact of early-stage MetS on coronary dilation to adenosine *in vivo* and *in vitro*, with particular focus on the role of A_2 receptors and K^+ channels, using a well established pig model of early-stage MetS (17). We examined the hypothesis that coronary dilation to adenosine is impaired in early-stage MetS via reduced A_2 channel function and/or diminished K^+ channel involvement.

Methods

Swine Model of Metabolic Syndrome. All protocols were approved by an Institutional Animal Care and Use Committee and complied with the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996). Adult male Ossabaw miniature swine (7–10 months old; gonadally intact) were assigned to two diet groups for 9 weeks. Lean control swine (Control, $n = 6$) were fed ~2200 kcal/day of standard chow (5L80, Purina TestDiet, Inc., Richmond, IN) containing 18% kcal from protein, 71% kcal from complex carbohydrates, and 11% kcal from fat. MetS ($n = 5$) was induced by feeding ~8000 kcal/day of high-fat/cholesterol/fructose diet containing 17% kcal from protein, 20% kcal from complex carbohydrates, 20% kcal from fructose, and 43% kcal from fat (lard, hydrogenated soybean oil, and hydrogenated coconut oil), supplemented with 2.0% cholesterol and 0.7% sodium cholate by weight (5B4L, Purina TestDiet, Inc., Richmond, IN). Given the short 9-week period of high-fat/cholesterol/fructose feeding and subsequent exposure to MetS symptoms, we consider this a model of early-stage MetS. Pigs were individually housed on a 12-hr light:dark cycle and provided water *ad libitum*. Before sacrifice (36–50 weeks of age), under isoflurane anesthesia, blood samples were drawn and serum glucose and cholesterol were determined in a blinded fashion (Antech Diagnostics, Fishers, IN).

Assessment of Coronary Artery Disease. Intravascular ultrasound (IVUS) was conducted on the left circumflex artery as previously described (12, 18). The IVUS catheter (3.2 F 35 MHz Ultracross or 2.9F 40 MHz Discovery, Boston Sci. Corp. on a Hewlett Packard Sonos console) was advanced ~40 mm down coronary arteries to obtain "serial sections" of ultrasound dimensions along the artery to quantify morphological changes indicative of atherosclerosis. We defined atherosclerosis as any fibrous or soft plaque less echogenic than the adventitia (19–21). Atheroma was quantified as percent of circumferential lumen wall coverage. Images were obtained every 1 mm through the length of the artery. Each cross-sectional IVUS image was divided into 16 equal radial segments, and percent circumferential wall coverage was calculated as (# radial segments containing atheroma ÷ 16) × 100%.

***In Vivo* Coronary Procedures.** The methods for these procedures were recently described in detail (11). Briefly, pigs were sedated with telazol (5 mg/kg) and xylazine (2.2 mg/kg) and anesthesia maintained with morphine sulfate (3 mg/kg, iv) and α-chloralose (100 mg/kg, iv). The left anterior descending (LAD) coronary artery was isolated and a perivascular Transonics flow transducer (2.5 mm) was placed around the artery. A 24-gauge angiocatheter was inserted into the LAD for intracoronary bolus injection of a moderate dose of adenosine (1 μg/kg). Following instrumentation, the adenosine bolus was delivered and the peak flow response measured.

Isolation of Coronary Arterioles. Following removal of the heart, myocardial sections from the apex and left ventricle were immediately placed in ice-cold physiological salt solution (PSS) consisting of (in mM): 145 NaCl, 4.7 KCl, 1.2 NaH_2PO_4, 1.17 $MgSO_4$, 2.0 $CaCl_2$, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, 3.0 MOPS, and 1% bovine serum albumin, pH 7.4. These samples were sent via airmail to the University of Missouri for *in vitro* experiments, similar to a previous study (12). Tissue was used within 24 hr from removal of the heart. Subepicardial coronary arterioles (50–150 μm) were isolated, cannulated with glass micropipettes, pressurized to 60 cmH_2O, visualized and measured as previously described (22). Arterioles were equilibrated for ~1 hr at 37°C with the bathing PSS solution always changed every 15 min, except during dose response measurements. Coronary arterioles ($n = 3$ from each animal) were preconstricted to 50–70% tone with endothelin-1 (ET-1) for examination of dilator responses.

***In Vitro* Experimental Protocols.** *In vitro* coronary arteriolar responses to adenosine were assessed using the adenosine analog 2-chloroadenosine (2-CAD; 0.1 nM–0.1 mM). This compound was utilized to avoid the potential impact that alterations in vascular nucleoside transport activity, via components of the MetS, may have on agonist levels at adenosine receptor beds (23, 24). Previous work demonstrated that dilation to 2-CAD was unaffected by nucleoside transport inhibition, whereas dilation to adenosine was enhanced in porcine coronary smooth muscle (25). Therefore, utilization of 2-CAD ensured that the effective

agonist concentration at the receptor beds was identical in both groups.

Role of Adenosine A_2 Receptors. The role of adenosine type A_{2A} and A_{2B} receptors in dilation to 2-CAD was examined using two arterioles from each animal. Concentration-dependent dilator responses of arterioles to 2-CAD was determined in each vessel by cumulative additions of 2-CAD (0.1 nM–0.1 mM) following ET-1 preconstriction. Vessels were then washed for 45 min with PSS and allowed to return to baseline diameter prior to pretreatment with the adenosine A_{2A} receptor antagonist ZM241385 (0.1 μM) or the A_{2B} receptor antagonist alloxazine (10 μM) for 30 min. 2-CAD responses were then repeated. Following a second wash, each vessel was pretreated with ZM241385 plus alloxazine for 30 min and 2-CAD responses repeated. Time controls for three consecutive 2-CAD dose-response measurements were performed in coronary arterioles from both groups ($n = 3$) with no evidence of tachyphylaxis to 2-CAD (data not shown).

Role of K^+ Channels. The role of K_{ATP} and K_v channels in arteriolar dilation to 2-CAD was examined by selective blockade with glibenclamide (GB; 10 μM) and 4-aminopyridine (4-AP; 1 mM), respectively. Concentration-dependent responses to 2-CAD (0.1 nM–0.1 mM) were determined, the vessel was washed as described above then pretreated with GB and 4-AP (in random order) for 30 min and 2-CAD responses repeated. Drugs were washed out for 45 min between dose-response measurements during which time vessels returned to baseline diameter.

Each vessel was then washed for 45 min and concentration-dependent responses to the nitric oxide (NO) donor sodium nitroprusside (SNP; 0.1 nM–0.1 mM) were determined. Maximum passive diameter was measured with the vessel bathed for 45 min in calcium-free PSS (2 mM $CaCl_2$ replaced with 2 mM EDTA) plus thapsigargin (10 μM), a SERCA inhibitor.

Immunoblot Analysis. The expression of adenosine A_{2A} and A_{2B} receptor protein in porcine coronary arterioles from both groups was assessed by immunoblot analysis. Coronary arterioles ($n = 5$ per tube, mean luminal diameter *in situ* ~100 μm, 1 mm in length) were prepared by digestion in 50 mM Tris·HCl, pH 7.4, containing 6 M urea, 150 mM dithiothreitol and 2% SDS by intermittent heating and agitation. Total protein content of tissue lysates was determined using a NanoOrange® protein quantitation kit (Invitrogen). Ten μg protein/lane were separated by 12% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. Gels included samples from both control and MetS swine. Positive controls for both receptor subtypes were conducted using rat brain (RB) extract (Stressgen; Fig. 1). Membranes were probed with specific anti-A_{2A} (1:1000 dilution; Alpha Diagnostic International, TX) or -A_{2B} (1:500 dilution; Chemicon International) receptor antibodies followed by incubation with secondary antibody conjugated to horseradish peroxidase. Antibody and blocking solutions contained 5% nonfat milk (A_{2A}) or bovine serum albumin (A_{2B}) and 0.1% Tween 20 in Tris-buffered saline. Protein expression was detected by enhanced chemiluminescence (SuperSignal® Extended Duration Substrate, Thermo Scientific).

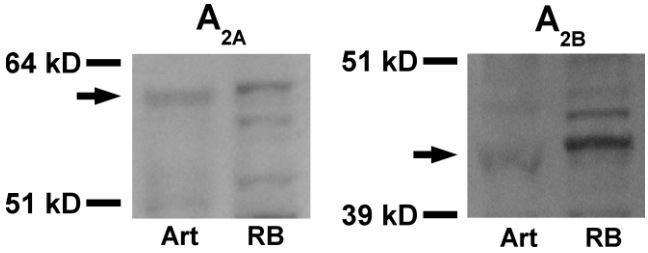

Figure 1. Representative positive control Western blot signals for adenosine A_2 receptor subtypes in porcine coronary arterioles (Art) and rat brain (RB) extract.

Drugs and Solutions. 2-CAD, GB, 4-AP, alloxazine and PSS components were purchased from Sigma (St. Louis, MO). ZM241385 was purchased from Tocris (Ellisville, MO). Stock solutions of 2-CAD and 4-AP were prepared in distilled water and frozen. GB, alloxazine and ZM241385 stock solutions were prepared in dimethylsulfoxide and frozen. On the day of the experiment, stock solutions were thawed and diluted in PSS to the appropriate working concentration. The final concentration of dimethylsulfoxide in the vessel bath was 0.1%. Vehicle control studies indicated no effect of this solvent concentration on arteriolar function.

Data Analysis. All *in vitro* arteriolar diameter changes in response to agonists were normalized to maximal passive diameter and presented as percent maximal dilation. Percent maximal dilation is the percent change in diameter relative to the maximal change in diameter possible calculated as $[(Dd - Db) / (Dmax - Db) \times 100]$, where Dd is diameter after a drug intervention, Db is baseline diameter, and Dmax is maximal diameter. The 2-CAD concentration that produced 50% of the maximal vasodilation was determined using Prism software and designated the EC_{50}. All EC_{50} values calculated were converted to log values for statistical comparison. Statistical analysis of concentration-response curves was performed using a one-way ANOVA for repeated measures followed by a least square mean analysis to determine differences in 2-CAD responses due to drug interventions within each group. Unpaired *t* test was used to test for between group differences in adenosine-induced coronary vasodilation *in vivo* and immunoblot results.

Results

Phenotypic Characteristics of Ossabaw Swine. Nine weeks of high-fat/cholesterol feeding in Ossabaw swine induced early-stage MetS characterized by obesity, hyperglycemia and hypercholesterolemia, confirming previous results (17). Body weights for control and MetS pigs were 42 ± 1 and 53 ± 6 kg, respectively ($P <$

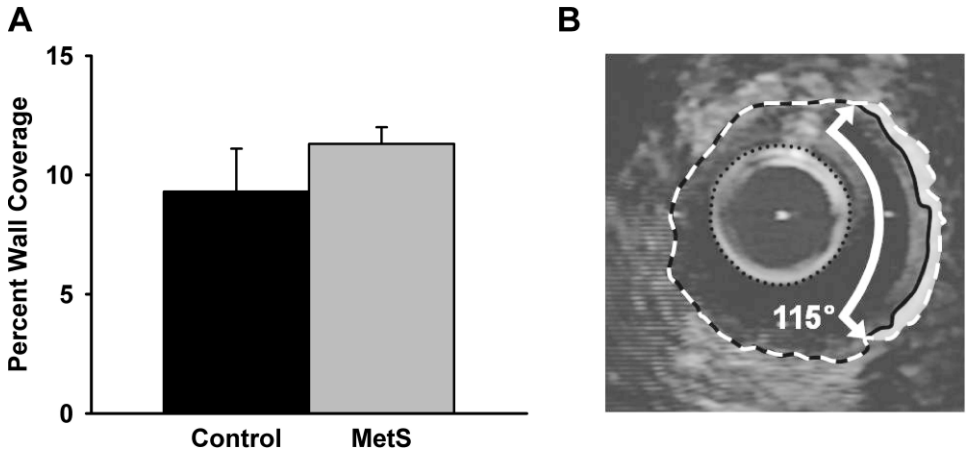

Figure 2. Quantification of coronary atheroma in left circumflex artery by intravascular ultrasound (IVUS). **A:** Coronary atheroma, expressed as percent wall coverage, in control ($n = 3$) and MetS ($n = 3$) swine. Values are mean ± SE. **B:** Example of an IVUS 'serial section' depicting the IVUS catheter (dotted black line), luminal border (solid black line), internal elastic lamina (dashed white line) and neointima (shaded area between solid black and dashed white lines). The white double arrow curve depicts the number of luminal degrees covered by neointima that is then converted to percent wall coverage.

0.05). Fasting blood glucose was 56 ± 13 mg/dl in control versus 114 ± 4 mg/dl in MetS pigs. Total cholesterol was 56 ± 19 and 234 ± 28 mg/dl in control and MetS pigs, respectively. There was no difference in mean aortic pressure (97 ± 5 vs 93 ± 9 mmHg; $n = 4$) or heart rate (71 ± 17 vs 92 ± 9 bpm; $n = 5$) measured under anesthesia for control and MetS swine, respectively. Analysis of serial coronary sections by IVUS revealed early circumferential neointimal hyperplasia in both groups; however, this was not different ($P = 0.38$) between groups (Fig. 2).

Coronary Microvascular Reactivity to Adenosine. *In vivo* coronary dilation to adenosine (1 μg/kg bolus) was examined in both control ($n = 4$) and MetS ($n = 5$) swine. Baseline coronary blood flow was similar between groups and infusion of adenosine significantly increased coronary blood flow similarly in both groups (Table 1) without altering systemic hemodynamics.

For *in vitro* arteriolar studies, maximal diameters of control and MetS cannulated arterioles at 60 cmH_2O intraluminal pressure were not different, respectively (107 ± 8 μm, $n = 16$; 107 ± 7 μm, $n = 13$). The level of preconstriction (% maximal intraluminal diameter) was similar between groups (69 ± 3% in control vs 68 ± 4% in MetS). The concentration of ET-1 necessary for preconstriction was also similar in control and MetS arterioles (4.9 ± 0.6 nM vs 5.3 ± 1.0 nM). We found that 2-CAD elicited similar dose-dependent increases in arteriolar diameter in both control and MetS swine (Fig. 3A). 2-CAD EC_{50} averaged 1.1 ± 4.2 μM in control and 4.6 ± 2.9 μM in MetS arterioles ($P = 0.26$). Maximal dilation to 2-CAD (% maximal diameter) was also similar between groups (87 ± 3% vs 85 ± 2%). Similar to adenosine, no differences in arteriolar dilation to the endothelium-independent vasodilator SNP were noted between groups (Fig. 3B).

Table 1. In Vivo CBF Response to Adenosine in Control and MetS Swine[a]

Parameter	Control ($n = 4$)	MetS ($n = 5$)	P value
Baseline CBF, ml/min/g	0.7 ± 0.02	0.7 ± 0.07	0.86
Peak CBF, ml/min/g	2.3 ± 0.4	1.9 ± 0.3	0.43
Delta flow, ml/min/g	1.6 ± 0.4	1.2 ± 0.2	0.36

[a] CBF, coronary blood flow. Values are mean ± SE.

Role of Adenosine A_2 Receptor Subtypes. Individual or combined inhibition of A_{2A} or A_{2B} receptors did not alter resting arteriolar tone in either group. In control arterioles, blockade of A_{2A} receptors with ZM241385 significantly blunted 2-CAD-induced dilation while A_{2B} inhibition with alloxazine did not alter the response (Fig. 4A). Correspondingly, combined $A_{2A/2B}$ inhibition reduced the response similarly to that with A_{2A} inhibition alone (Fig. 4A). In MetS arterioles, selective inhibition of A_{2A} or A_{2B} receptors had no significant effect on dilation to 2-CAD (Fig. 4B). Combined A_{2A} and A_{2B} inhibition, however, caused a significant rightward shift of the 2-CAD curve (Fig. 4B).

Role of K_{ATP} and K_v Channels. Selective blockade of K_{ATP} channels with GB and K_v channels with 4-AP did not alter resting arteriolar tone in either group. In control arterioles, inhibition of K_{ATP} channels reduced arteriolar sensitivity ($EC_{50} = 11 \pm 6$ μM; $P = 0.058$) and maximal dilation to 2-CAD ($P = 0.054$). K_v channel inhibition had a similar effect in reducing arteriolar sensitivity to 2-CAD ($EC_{50} = 12 \pm 4$ μM; $P < 0.05$) but did not reduce the maximal dilator response (Fig. 5A). In MetS arterioles, K_{ATP} channel inhibition did not alter arteriolar dilation to 2-CAD ($EC_{50} = 6 \pm 5$ μM; $P = 0.55$). Conversely, inhibition of K_v channels reduced arteriolar sensitivity to 2-CAD ($EC_{50} = 39 \pm 28$ μM; $P < 0.05$) but did not affect maximal dilation (Fig. 5B).

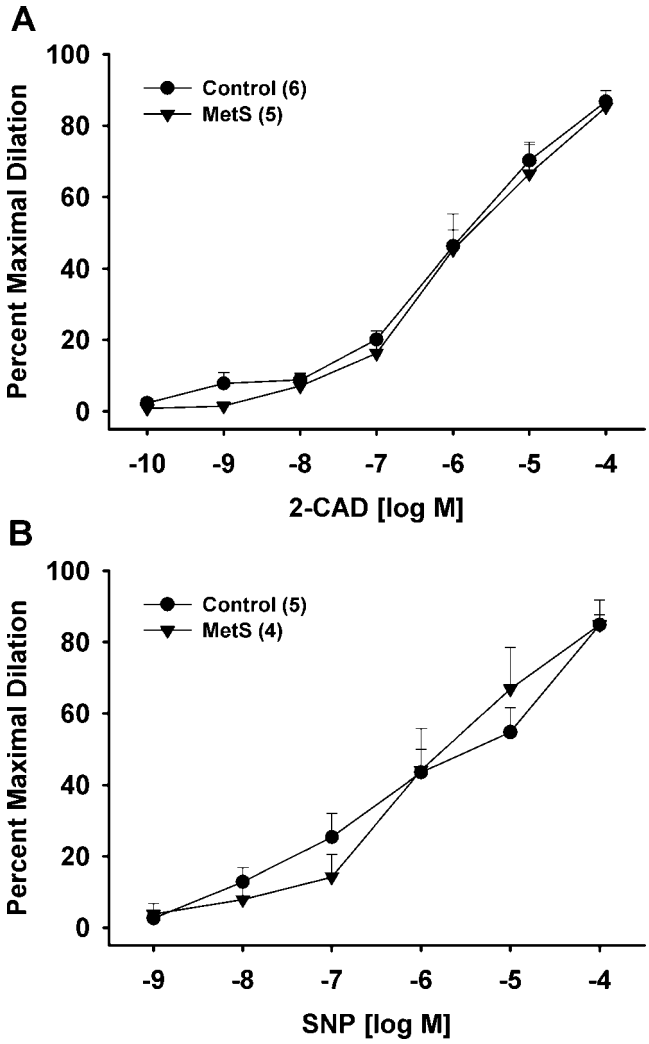

Figure 3. Concentration-response curves of coronary arterioles from control and MetS pigs to 2-chloroadenosine (**A**; 2-CAD) and sodium nitroprusside (**B**; SNP). Values are mean ± SE, sample size in parentheses.

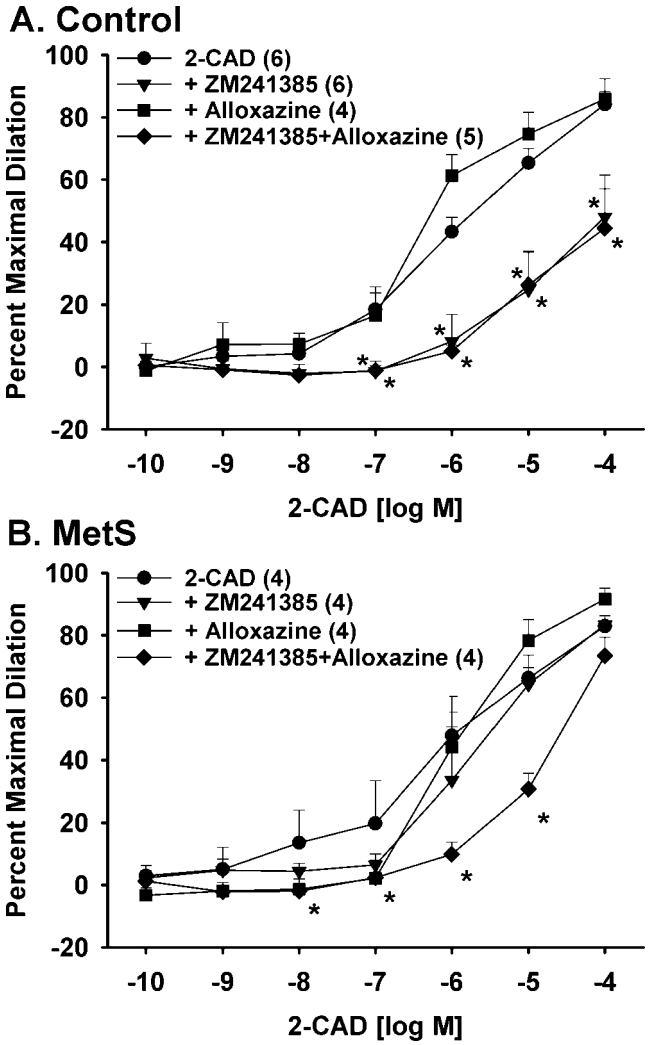

Figure 4. Effect of adenosine A_{2A} receptor inhibition with ZM241385 and A_{2B} receptor inhibition with alloxazine on 2-CAD-mediated dilation of coronary arterioles from control (**A**) and MetS (**B**) pigs. Values are mean ± SE, sample size in parentheses represents the number of paired dose-response measurements to 2-CAD made with each drug treatment. * $P < 0.05$ versus control in either group.

Immunoblot Analysis of Adenosine A_2 Receptor Subtypes. Immunoblot analysis revealed adenosine A_{2A} (~60 kDa) receptor protein expression in porcine coronary arterioles, similar to previous reports (6, 26). Expression of adenosine A_{2B} receptors (~42 kDa) was also demonstrated in coronary arterioles within its previously reported molecular weight range of 34–55 kDa (26–28). Expression of A_{2B}, but not A_{2A}, receptor protein in coronary arterioles was reduced by early-stage MetS (Fig. 6).

Discussion

The onset of MetS and its subsequent complications occur progressively over long periods of time, significantly increasing the risk of death, particularly via coronary vascular disease (1). To date, however, little is known about the effect of early-stage MetS on the coronary microcirculation and the mechanisms of coronary blood flow control. The experiments presented herein were designed to test the hypothesis that early-stage MetS reduces adenosine-induced dilation in the coronary microvasculature through reduced adenosine A_2 receptor function and/or attenuated downstream K^+ channel function. Nine weeks of high-fat/fructose/cholesterol diet feeding in Ossabaw swine induced early-stage MetS, as previously reported (17). Coronary atheroma was negligible and not different between groups as expected in early-stage MetS. Thus, early-stage MetS is associated almost exclusively with microvascular disease and is not confounded by flow-limiting macrovascular coronary stenosis. The primary findings of this study are: i) coronary arteriolar dilation to 2-CAD in control vessels is mediated primarily by A_{2A} receptors, in contrast both A_{2A} and A_{2B} receptors are involved in dilation of MetS arterioles; ii) arteriolar

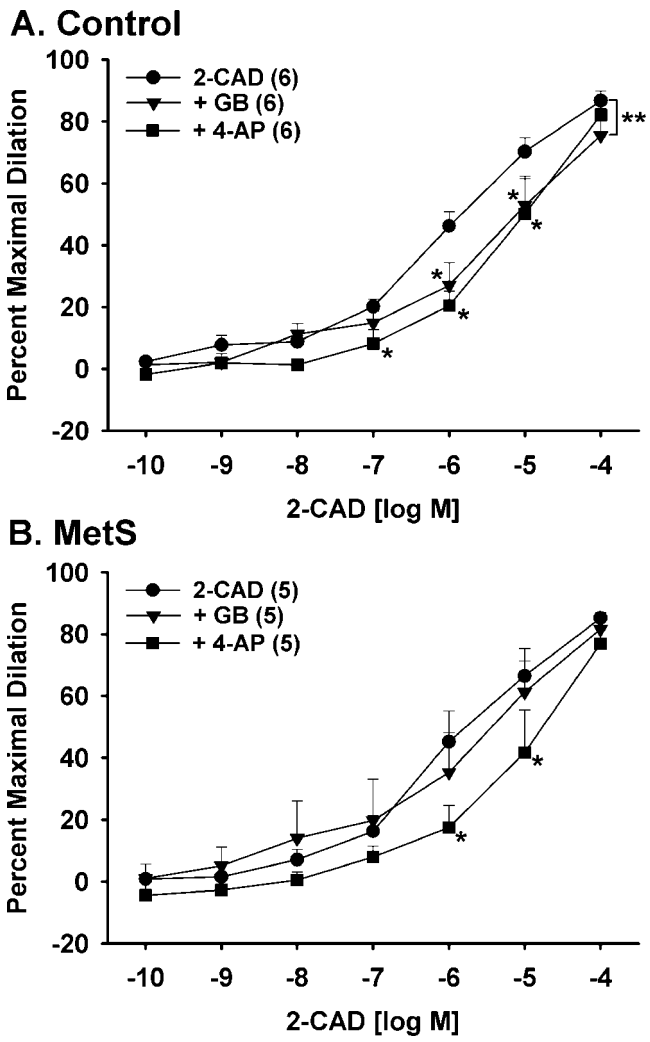

Figure 5. Effect of K_v channel inhibition with 4-AP and K_{ATP} channel inhibition with GB on 2-CAD-mediated dilation of coronary arterioles from control (**A**; $n = 6$) and MetS (**B**; $n = 5$) pigs. Values are mean ± SE, sample size in parentheses represents the number of paired dose-response measurements to 2-CAD made with each drug treatment. * $P < 0.05$ versus control (within group); ** $P = 0.054$ versus control.

expression of A_{2B}, but not A_{2A}, receptor protein is reduced by early-stage MetS; iii) K_{ATP}, but not K_v, channel involvement in dilation to 2-CAD is abolished by MetS; but iv) adenosine- and 2-CAD-induced dilation of coronary arterioles was maintained in early-stage MetS *in vivo* and *in vitro,* respectively. Therefore, our data indicate that the functional contribution of A_2 receptors and K_{ATP} channels to coronary microvascular dilation is significantly altered in early-stage MetS while overall adenosine responsiveness is not yet altered.

Adenosine plays an important role in the control of coronary blood flow, particularly during episodes of cardiac ischemia (3, 4). The coronary vasodilator response to adenosine (i.e., coronary flow reserve) is used clinically as an indicator of microvascular function. It was recently demonstrated that patients with established MetS have reduced coronary flow reserve in response to infusion of the adenosine uptake inhibitor dipyridamole (5). The more advanced progression of MetS in these patients likely explains the apparent difference with our findings that early-stage MetS did not alter coronary flow reserve in response to exogenous adenosine *in vivo* or 2-CAD-mediated dilation of isolated coronary arterioles in swine. Our findings demonstrate significant early-stage alterations in the mechanism of coronary microvascular vasodilation to adenosine, which may precede overt decreases in coronary flow reserve in the MetS.

Role of A_2 Receptor Subtypes. Previous studies have demonstrated that coronary microvascular dilation to adenosine operates primarily through activation of A_{2A} receptors (6, 9, 29). Reports in human small coronary arteries (7) and isolated rat hearts (30) suggest a principal role for A_{2B} receptors. Selective A_{2B} antagonism was used only in the latter study (30). Expression of adenosine A_1, A_{2A} and A_{2B} receptors is established in the coronary microcirculation (6, 26, 29). We examined whether early-stage MetS alters the expression and/or role of A_2 receptor subtypes responsible for coronary arteriolar dilation to adenosine. To that end, we demonstrate, as others have, that activation of A_{2A} receptors is the primary initiator of

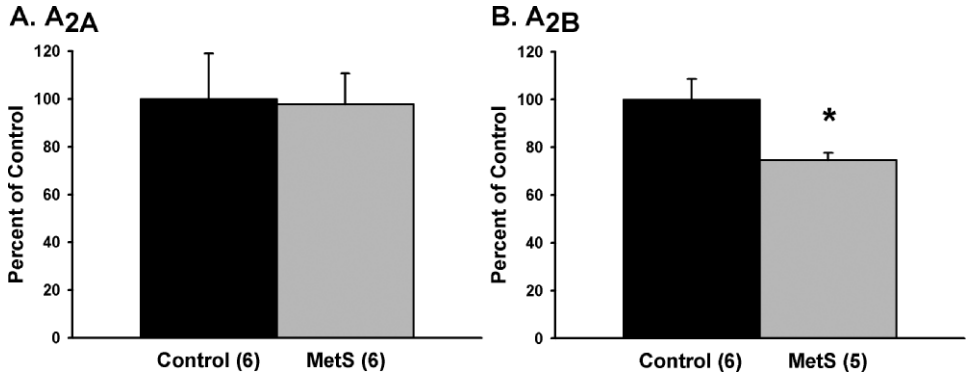

Figure 6. Immunoblot analysis of adenosine A_{2A} (**A**) and A_{2B} (**B**) receptors in coronary arterioles from control and MetS pigs. Values are percent of control protein expression ± SE, sample size in parentheses (5 pooled arterioles per animal in each sample). * $P < 0.05$ versus control.

adenosine-mediated vasodilation in the coronary microcirculation of swine (6, 8). Further, selective inhibition of A_{2B} receptors alone or in combination with A_{2A} inhibition had no additional inhibitory effect on 2-CAD-mediated vasodilation in normal arterioles, suggesting no significant A_{2B} receptor involvement.

The impact of early-stage MetS on the receptors mediating microvascular dilation to adenosine has not been previously examined. Various studies have demonstrated diabetes-related alterations in protein expression of adenosine A_2 receptor subtypes in various tissues (i.e., heart, kidney, liver, hippocampus) (31–34). Here we demonstrate for the first time that coronary arteriolar A_{2B}, but not A_{2A}, receptor protein expression is reduced by early-stage MetS. It remains uncertain, however, whether this change in expression occurs in the endothelium or smooth muscle since these receptors are expressed in both cell types. Functionally, our results indicate that, in contrast to control arterioles, both A_{2A} and A_{2B} receptors are independently capable of mediating arteriolar dilation to 2-CAD in early-stage MetS such that only combined $A_{2A/2B}$ inhibition reduced this response. In light of the reduced A_{2B} expression in early-stage MetS, the increased role for A_{2B} receptors in mediating 2-CAD-induced dilation could be attributed to enhanced receptor sensitivity and/or coupling to intracellular signaling cascades. This remains to be determined. To our knowledge, these are the first data to demonstrate that altered adenosine A_2 receptor subtype vasodilator function is an early microvascular adaptation to MetS.

Combined $A_{2A/2B}$ inhibition did not reduce maximal 2-CAD dilation in early-stage MetS arterioles. Maximal dilation appears to be maintained in early-stage MetS arterioles through an A_2 receptor-independent mechanism. Activation of A_1 receptors has been shown to attenuate adenosine dilation of coronary vessels from dogs (35), mice (36) and humans (29). Studies in pigs (26) and humans (29) demonstrate A_1 receptor mRNA and protein in coronary arterioles; however, Hein *et al*. (6) suggest that these receptors are not functionally significant in porcine microvessels since A_1 receptor blockade did not alter arteriolar dilation to adenosine. In addition, indirect evidence suggests that A_3 receptor activation contributes to coronary dilation to adenosine in the isolated rat heart (30). The presence of A_3 receptor mRNA, but not protein, has been demonstrated in pig coronary microvessels (26). Taken together, it is possible that either reduced A_1-mediated inhibition of adenosine dilation or enhanced A_3-mediated dilation may account for the maintenance of maximal arteriolar dilation to adenosine in early-stage MetS. Further studies are necessary to fully delineate the mechanistic role of A_1 and A_3 receptor subtypes in coronary microvascular dilation to adenosine and the potential influence of early-stage MetS to alter the function of and interaction between adenosine receptors.

Involvement of K^+ Channels. The opening of various K^+ channels, particularly K_{ATP} and K_v, is a primary mechanism through which adenosine acts to hyperpolarize and relax microvascular smooth muscle (6, 9, 10). In a comprehensive examination of K^+ channel involvement in this response, Heaps *et al*. (10) demonstrated that coronary dilation to adenosine in pig arterioles is sensitive to inhibition of K_v, but not K_{ATP} or K_{Ca}, channels. Recently, Dick *et al*. (11) confirmed a role for K_v channels in coronary arteriolar dilation to adenosine *in vitro* and *in vivo* in the canine heart. In contrast, Hein *et al*. (6, 9) demonstrate that this response in pig arterioles is entirely dependent on the activation of K_{ATP} channels by adenosine. Likewise, *in vivo* studies have demonstrated a K_{ATP} channel-dependent component of adenosine-mediated coronary dilation in humans and dogs (37, 38). Our data are consistent with both sets of observations illustrating a role for both K_{ATP} and K_v channels in coronary arteriolar dilation to adenosine in normal porcine coronary arterioles.

We report the novel finding that early-stage MetS differentially affects K^+ channel involvement in coronary dilation to adenosine through an abolished contribution of K_{ATP} channels with no effect on K_v channels. This result was surprising in light of abundant data that diabetes, hypercholesterolemia and acute hyperglycemia can reduce K_v channel function in the coronary microcirculation (13, 15, 16). Our results are consistent, however, with previous work demonstrating reduced dilation to K_{ATP} channel openers in aorta, cerebral arteries and skeletal muscle arterioles of diabetic rats, as well as coronary arterioles from patients with type 2 diabetes (14, 39–41). This was also recently demonstrated in skeletal muscle arterioles from the obese Zucker rat (41). In addition, pilot results from our lab demonstrate a trend toward reduced coronary arteriolar dilation to the K_{ATP} channel opener levcromakalim in early-stage MetS (unpublished observations). Conversely, two studies have noted enhanced K_{ATP}-mediated vasodilation *in vivo* in coronary arterioles of acutely diabetic and hyperglycemic dogs (42, 43). The reason for this discrepancy is unclear but may be due to differences in species, length of disease, combination of risk factors or method of disease induction. The possibility of interaction between various K^+ channels in regulating arteriolar tone in MetS also cannot be excluded. Further studies using multiple K^+ channel blockade are necessary to address this issue. Whatever the reason for the difference between these and the present results, our data indicate that early-stage MetS presents a combination of stimuli which preferentially decreases the activity of K_{ATP} channels in the porcine coronary microvasculature.

While dysfunctional K_{ATP} channels may account for their reduced role in dilation to adenosine, the alternative that intracellular coupling between adenosine receptors and K_{ATP} channels is reduced cannot be excluded. Numerous studies have demonstrated that the endothelium-independent component of adenosine-mediated vasodilation occurs partly through cAMP-dependent activation of protein kinase A (PKA) and that these pathways are reduced by components of the MetS (15, 29, 44–46). It has been

demonstrated, however, that adenosine-mediated activation of K_{ATP} channels occurs through a cAMP-independent pathway in the pig coronary microcirculation (6, 9). Likewise, the lack of effect of early-stage MetS on the role of K_v channel activation by 2-CAD in this study, which is cAMP-dependent, argues against defective cAMP-PKA signaling in this model (47). Thus, our data are more consistent with reduced K_{ATP} channel function in early-stage MetS rather than reduced channel activation via adenosine receptors.

Limitations. The A_{2A} selective antagonist ZM241385 was utilized in the present study at a dose of 0.1 μM based on previous studies in porcine coronary arterioles (6, 48). It should be noted, however, that at this dose ZM241385 may also interact with A_{2B} receptors ($K_i = 33.6$ nM) (49). The lack of effect of the A_{2B} antagonist alloxazine on arteriolar dilation to 2-CAD in control arterioles, however, suggests that any impact on A_{2B} receptors was modest. In addition, at the doses used, both ZM241385 and alloxazine may antagonize A_1 receptors (50, 51). This possibility warrants further examination; however, previous studies in porcine coronary arterioles suggest that, while expressed in this tissue, A_1 receptors may be functionally inactive in adenosine-mediated dilation (i.e., A_1 antagonism did not affect adenosine dilation) (6, 26, 48).

Clinical Implications. Detection of coronary microvascular disease in the MetS is essential for appropriate treatment, especially since microvascular angina is common in this patient population (52–54). Clinically, adenosine stress testing is used to examine for myocardial perfusion defects at the microvascular level. Our data demonstrate, however, that coronary arteriolar responses to adenosine are maintained in early-stage MetS although defects in adenosine receptor activity and/or signaling exist. Thus, the detection of microvascular dysfunction in the early stages of MetS may be better accomplished using selective adenosine receptor agonists (i.e., A_{2A} or A_{2B}). In addition to their diagnostic value, agonists such as the recently described A_{2A} receptor agonist regadenoson demonstrate fewer side effects compared to adenosine or dipyridamole (55). Examination of the available literature in conjunction with our data also suggests that myocardial stress testing using a K_{ATP} channel opener may be more beneficial in detecting early arteriolar dysfunction in the MetS. Reduced function of vascular K_{ATP} channels is a common defect in coronary, cerebral and skeletal muscle arterioles in animal and human MetS/diabetes, although no study has examined temporal changes in channel function in disease (14, 39–41). Thus, it is imperative that as our understanding of the disease processes involved in MetS advances, so too does our ability to detect and treat these defects.

Conclusions. The present study demonstrates that early-stage MetS induces substantial alterations in the pathways mediating coronary microvascular dilation to adenosine although the overall response to adenosine is maintained. Our data suggest that A_2 receptor mediation of adenosine-induced coronary arteriolar dilation is significantly altered in early-stage MetS. Furthermore, we demonstrate that early-stage MetS attenuates K_{ATP} channel involvement in adenosine-mediated microvascular dilation. Therefore, these results demonstrate early changes in coronary microvascular function prior to the onset of overt decrements in coronary flow reserve in the MetS and may be important for our understanding of the deleterious effects of MetS on coronary blood flow control.

The authors gratefully acknowledge the assistance of Dr. Douglas Bowles.

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