Contribution of BK$_{Ca}$ channels to local metabolic coronary vasodilation: effects of metabolic syndrome

Léna Borbouse,1 Gregory M. Dick,2 Gregory A. Payne,1 Brittany D. Payne,1 Mark C. Svendsen,3 Zachary P. Neeb,1 Mouhamad Aloosh,1 Ian N. Bratz,4 Michael Sturek,1 and Johnathan D. Tune1

1Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, Indiana; 2Department of Exercise Physiology, Center for Cardiovascular and Respiratory Sciences, West Virginia University School of Medicine, Morgantown, West Virginia; 3Weldon School of Biomedical Engineering, Purdue University, West Lafayette, Indiana; and 4Department of Integrative Medical Sciences, Northeastern Ohio Universities Colleges of Medicine, Rootstown, Ohio

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This investigation was designed to examine the hypothesis that impaired function of coronary microvascular large-conductance Ca$^{2+}$-activated K$^+$ (BK$_{Ca}$) channels in metabolic syndrome (MetS) significantly attenuates the balance between myocardial oxygen delivery and metabolism at rest and during exercise-induced increases in myocardial oxygen consumption (MVO$_2$). Studies were conducted in conscious, chronically instrumented Ossabaw swine fed a normal maintenance diet (11% kcal from fat) or an excess calorie atherogenic diet (43% kcal from fat, 2% cholesterol, 20% kcal from fructose) that induces many common features of MetS. Data were collected under baseline/cardiac function or heart rate were noted. Induction of MetS produced a parallel downward shift in the relationship between coronary venous PO$_2$ and MVO$_2$ (P < 0.001) that was accompanied by decreases in BK$_{Ca}$ channel function (3, 4, 27, 34–36, 38). In particular, we found that the functional expression of BK$_{Ca}$ channels is markedly depressed in Ossabaw swine with MetS (3, 38). Because BK$_{Ca}$ channels have been shown to contribute to coronary endothelial-dependent and exercise-induced dilation under normal-lean conditions (3, 4, 27, 34–36, 38), we propose that decreases in BK$_{Ca}$ channel function could underlie impaired metabolic control of coronary blood flow in MetS. However, no study has examined the contribution of BK$_{Ca}$ channels to metabolic coronary vasodilation in the setting of MetS.

Accordingly, the goal of this investigation was to examine the hypothesis that impaired function of coronary microvascular BK$_{Ca}$ channels in MetS (3, 38) significantly attenuates the balance between myocardial oxygen delivery and metabolism at rest and during exercise-induced increases in MVO$_2$, i.e., metabolic coronary vasodilation. Studies were conducted in conscious, chronically instrumented Ossabaw swine fed a normal maintenance diet (11% kcal from fat) or an excess calorie atherogenic diet (43% kcal from fat, 2% cholesterol, 20% kcal from fructose) that induces many common features of MetS, including obesity, insulin resistance, impaired glucose tolerance, and dyslipidemia (3, 5, 18, 48). Coronary blood flow data were recorded, and arterial and coronary venous blood samples were collected before and after selective blockade of BK$_{Ca}$ channels with penitrem A (10 μg/kg iv) under baseline/resting conditions and during graded treadmill exercise up to ~75% of maximum whole body VO$_2$ (heart rate >200/min).

METHODS

Swine model of MetS. All experimental procedures and protocols used in this investigation were approved by the Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals. Lean control swine were fed ~2,200

DUE TO THE LIMITED ANAEROBIC capacity of the myocardium, the heart depends on a continuous supply of oxygen from the coronary circulation to meet its metabolic requirements (49). Thus, under normal physiological conditions, myocardial oxygen delivery is closely matched with the rate of myocardial oxidative metabolism. To ensure adequate balance between coronary blood flow and myocardial metabolism, powerful regulatory mechanisms exist to increase nutritive blood flow to the heart whenever myocardial oxygen consumption (MVO$_2$) is elevated (13, 49). However, despite decades of research, the exact mechanisms responsible for local metabolic control of coronary blood flow have yet to be clearly defined (49).

Previous studies have demonstrated that disease states such as obesity and metabolic syndrome (MetS) significantly impair control of coronary blood flow at rest and during increases in MVO$_2$ (7, 11, 30, 44, 46, 54). Coronary dysfunction in the MetS is characterized by an imbalance between coronary blood flow and myocardial metabolism (46) which has been attributed to sensitization of key vasoconstrictor pathways such as ANG II and α$_1$-adrenoceptors (29). Recently, data from our laboratory, as well as others, also established that obesity, insulin resistance, and type 2 diabetes diminishes end-effector mechanisms that regulate coronary vasodilation (3, 6, 9, 33, 38). In particular, we found that the functional expression of coronary large-conductance Ca$^{2+}$-activated K$^+$ (BK$_{Ca}$) channels is markedly depressed in Ossabaw swine with MetS (3, 38). Because BK$_{Ca}$ channels have been shown to contribute to coronary endothelial-dependent and exercise-induced dilation under normal-lean conditions (3, 4, 27, 34–36, 38), we propose that decreases in BK$_{Ca}$ channel function could underlie impaired metabolic control of coronary blood flow in MetS. However, no study has examined the contribution of BK$_{Ca}$ channels to metabolic coronary vasodilation in the setting of MetS.

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Swine model of MetS. All experimental procedures and protocols used in this investigation were approved by the Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals. Lean control swine were fed ~2,200

Address for reprint requests and other correspondence: J. D. Tune, Dept. of Cellular and Integrative Physiology, Indiana Univ. School of Medicine, 635 Barnhill Dr., Indianapolis, IN 46202 (e-mail: juone@iupui.edu).
kcal/day of standard chow (5L80; Purina, Richmond, IN) containing 18% kcal from protein, 71% kcal from complex carbohydrates, and 11% kcal from fat. MetS swine were fed an excess ~8,000 kcal/day high-fat/fructose, atherogenic diet containing 17% kcal from protein, 20% kcal from complex carbohydrates, 20% kcal from fructose, and 43% kcal from fat (mixture of lard, hydrogenated soybean oil, and hydrogenated coconut oil), and supplemented with 2.0% cholesterol and 0.7% sodium chloride by weight (H46T-F20–5L80; Purina, Richmond, IN). Both lean (n = 6 male) and MetS (n = 5 male) swine were fed their respective diets for 20 wk.

Surgical instrumentation and intravascular ultrasound. Ossabaw swine were fasted overnight before surgery. The animals were initially sedated with telazol (5 mg/kg sc) and xylazine (2.2 mg/kg sc). After endotracheal intubation, a surgical plane of anesthesia was maintained by mechanical ventilation with 1–3% isoflurane gas with supplemental oxygen. Using a sterile technique, a 7-Fr vascular introducer sheath (Boston Scientific) was inserted in the right femoral artery, and a guiding catheter (Amplatz L, sizes 0.75–2.0; Boston Scientific) was advanced to engage the left main coronary ostium. A 3.2 Fr, 30-MHz intravascular ultrasound (IVUS) catheter (Boston Scientific) was advanced over a guide wire and positioned in the coronary artery. Automated IVUS pullbacks were performed at 0.5 mm/s to obtain artery diameters, severity of atherosclerosis, and plaque morphology. Video images were analyzed off-line (Sonos Intravascular Imaging System; Hewlett Packard) (19).

Following the IVUS procedure, a left lateral thoracotomy was performed in the fifth intercostal space. A catheter (17-gauge pressure-monitoring catheter; Edwards Lifesciences) was implanted in the descending thoracic aorta to measure aortic blood pressure and to obtain arterial blood samples. A second catheter was placed in the coronary interventricular vein for coronary venous blood sampling and intravenous drug infusions. The left anterior descending (LAD) coronary artery was dissected free, and a Transonics perivascular flow transducer was placed around the artery. A chest tube was placed to evacuate the pneumothorax, and the chest was closed in layers. The transducer was placed around the artery. A chest tube was placed to evacuate the pneumothorax, and the chest was closed in layers. The catheters and the flow transducer wire were tunneled subcutaneously and exteriorized between the scapulas. Antibiotics (cephalexin) and aspirin (81 mg) were administered two times daily for 7 days. A jacket was placed on the animals to protect the catheters and the flow transducer wire. An elastomeric balloon pump (Access Technologies) was placed on the animals to protect the catheters and the flow transducer wire. A chest tube was placed to evacuate the pneumothorax, and the chest was closed in layers.

Surgical instrumentation and intravascular ultrasound. Ossabaw swine in a sternally recumbent position in a low-stress environment, blood pressure and heart rate were continuously recorded while the pigs were resting upright on the treadmill and then during the following two levels of treadmill exercise: 1) ~2 mph at 0% grade and 2) ~4 mph at 5% grade. Both lean and MetS swine exercised at similar intensity levels. Arterial and coronary venous blood samples were collected simultaneously in heparinized saline (5,000 U/ml) (45, 46, 54).

Echocardiographic studies. Two-dimensional and M-mode images were obtained from conscious lean (n = 4) and MetS (n = 4) Ossabaw swine in a sternal recumbent position in a low-stress restraint sling (41) using a Phillips IE33 echocardiography system (31). Images were obtained from a left parasternal approach at the midpapillary muscle level and recorded on the imaging system. Fractional shortening measurements were made using criteria from the American Society of Echocardiography (43). Measurements of the end-diastolic dimension (EDD) and end-systolic dimension (ESD) were averaged over five beats. EDD was obtained at the onset of the QRS complex, and ESD was at the end of the T wave. The left parasternal view was used to obtain aortic diameters at the root of the aortic valve during the T wave peak or at the instant of maximum dilation. Pulsed Doppler was used to measure blood velocity through the aorta. LV function was assessed using fractional shortening ([EDD – ESD]/EDD) × 100) and cardiac output (SV × HR), where SV is stroke volume and HR is heart rate.

Experimental protocol. Following recovery from surgery, experiments were conducted in lean (n = 6) and MetS (n = 5) Ossabaw swine before and after inhibition of BKCa channels with penitrem A (10 µg/kg iv) under baseline/resting conditions and during graded treadmill exercise up to ~75% of maximum whole body VO₂ (heart rate >200/min). We previously demonstrated that this intravenous dose of penitrem A essentially abolished coronary vasodilation to the BKCa channel agonist NS-1619 in anesthetized, open-chest lean Ossabaw swine (3). Coronary blood flow, aortic pressure, and heart rate were continuously recorded while the pigs were resting upright on the treadmill and then during the following two levels of treadmill exercise: 1) ~2 mph at 0% grade and 2) ~4 mph at 5% grade. Both lean and MetS swine exercised at similar intensity levels. Arterial and coronary venous blood samples were collected simultaneously in heparinized syringes when hemodynamic variables were stable at rest and at each level of exercise. Each exercise period was ~2 min in duration, and the animals were allowed to rest sufficiently between each level for hemodynamic variables to return to baseline.

Blood sampling. Arterial and coronary venous blood samples were collected, immediately sealed, and placed on ice. The samples were analyzed in duplicate for pH, PCO₂, PO₂, glucose, hematocrit, and oxygen content with an Instrumentation Laboratories automatic blood gas analyzer (GEM Premier 3000) and CO-oximeter (682) systems. LAD perfusion territory was estimated to be 30% of total heart weight, as previously described by Feigl et al. (21). MV O₂ (µl O₂·min⁻¹·g⁻¹) was calculated by multiplying coronary blood flow by the coronary arterial-venous difference in oxygen content. Lactate

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### Table 1. Phenotypic characteristics of lean and metabolic syndrome Ossabaw swine

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>MetS</th>
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</thead>
<tbody>
<tr>
<td>Body wt, kg</td>
<td>58 ± 6</td>
<td>77 ± 5*</td>
</tr>
<tr>
<td>Heart wt/body wt ×100</td>
<td>0.41 ± 0.01</td>
<td>0.39 ± 0.05</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>71 ± 4</td>
<td>96 ± 7*</td>
</tr>
<tr>
<td>Fasting insulin, µU/ml</td>
<td>10 ± 1</td>
<td>31 ± 4*</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>59 ± 4</td>
<td>383 ± 44*</td>
</tr>
<tr>
<td>LDL-to-HDL ratio</td>
<td>0.8 ± 0.1</td>
<td>3.4 ± 0.4*</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>22 ± 2</td>
<td>67 ± 6*</td>
</tr>
</tbody>
</table>

Values are means ± SE for lean (n = 6) and metabolic syndrome (MetS; n = 5) swine. LDL, low density lipoprotein; HDL, high density lipoprotein. *P < 0.05 vs. lean.
Table 2. *K* CHANNELS IN METABOLIC CORONARY VASODILATION

Table 2—Continued

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Rest</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary venous Po&lt;sub&gt;2&lt;/sub&gt;, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>16 ± 0.8</td>
<td>15 ± 0.8</td>
<td>15 ± 0.8</td>
</tr>
<tr>
<td>Lean + penitrem A</td>
<td>18 ± 0.8</td>
<td>16 ± 0.8</td>
<td>15 ± 0.8*</td>
</tr>
<tr>
<td>MetS</td>
<td>13 ± 0.9</td>
<td>12 ± 1.0†</td>
<td>13 ± 1.0</td>
</tr>
<tr>
<td>MetS + penitrem A</td>
<td>13 ± 0.9</td>
<td>14 ± 0.9</td>
<td>15 ± 0.9</td>
</tr>
<tr>
<td>Coronary venous O&lt;sub&gt;2&lt;/sub&gt; saturation, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>19 ± 2</td>
<td>16 ± 2</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>Lean + penitrem A</td>
<td>24 ± 2†</td>
<td>20 ± 2*</td>
<td>17 ± 2*</td>
</tr>
<tr>
<td>MetS</td>
<td>15 ± 2†</td>
<td>15 ± 2</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>MetS + penitrem A</td>
<td>16 ± 2</td>
<td>17 ± 2</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Arterial hematocrit, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>32 ± 2</td>
<td>37 ± 3</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>Lean + penitrem A</td>
<td>33 ± 3</td>
<td>35 ± 3</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>MetS</td>
<td>31 ± 3</td>
<td>35 ± 2</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>MetS + penitrem A</td>
<td>30 ± 2</td>
<td>33 ± 3</td>
<td>34 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE for lean (n = 6) and MetS (n = 5) swine. *P < 0.05 vs. respective baseline (rest) (*) and vs. lean same level (†).

uptake (μmol·min<sup>-1</sup>·g<sup>-1</sup>) was calculated by multiplying coronary blood flow by the coronary arterial-venous difference in lactate concentration.

Statistical analyses. Data are presented as means ± SE. Statistical comparisons were made with *t*-tests and three-way repeated-measures ANOVA (factor A: diet; factor B: drug treatment; factor C: exercise level) as appropriate. In all statistical tests, *P < 0.05 was considered statistically significant. When significance was found with ANOVA, a Student-Newman-Keuls multiple-comparison test was performed to identify differences between groups and treatment levels. Linear regression analysis was used to compare slopes of response variables (aortic pressure, heart rate, coronary venous Po<sub>2</sub>, lactate uptake) plotted vs. MV<sub>O</sub>2. If the slopes of the regression lines were not significantly different, an analysis of covariance was used to adjust response variables for linear dependence on MV<sub>O</sub>2.

RESULTS

Phenotype of *Ossabaw* swine. Phenotypic characteristics of lean and MetS swine are given in Table 1. We found that 20 wk of an excess calorie atherogenic diet induced classic features of MetS in Ossabaw swine. In particular, relative to their lean counterparts, MetS swine exhibited a 1.3-fold increase in body weight, 1.4-fold increase in fasting glucose, 3.1-fold increase in fasting insulin, 6.5-fold increase in total cholesterol, a 4.3-fold increase in the low density lipoprotein-to-high density lipoprotein ratio, and a 3-fold increase in triglyceride levels. MetS induced a fourfold increase in coronary atherosclerosis wall coverage of the LAD and a significant threefold increase in percent stenosis (nonflow limiting) relative to lean (Fig. 1).

Effects of MetS on coronary and cardiovascular responses to exercise. Hemodynamic and blood gas data at rest and during exercise for the lean and MetS *Ossabaw* swine before and after inhibition of BK<sub>Ca</sub> channels are summarized in Table 2. Systolic, diastolic, and mean aortic pressure were not different between lean and MetS swine under baseline resting conditions. Importantly, however, exercise-induced increases in aortic pressure were exaggerated in MetS vs. lean swine (Table 2 and Fig. 2A). No differences in heart rate were noted between groups at rest or during exercise (Fig. 2B).

Coronary blood flow and MV<sub>O</sub>2 were elevated approximately twofold in both lean and MetS swine at the highest level.
of exercise. The relationship between coronary venous PO₂ and MV̇O₂ (Table 2 and Fig. 3A), a sensitive index of myocardial tissue oxygenation that reflects whether changes in myocardial oxygen delivery adequately match myocardial metabolism (49), revealed a parallel downward shift in coronary venous PO₂ at a given level of MV̇O₂ in MetS swine (P < 0.001). The impairment of myocardial oxygen supply-demand balance in MetS swine was accompanied by significant decreases in myocardial lactate uptake with exercise-induced increases in MV̇O₂ (P < 0.005; Fig. 3B). The net release of lactate across the coronary circulation of MetS swine, which indicates the onset of anaerobic glycolysis and cardiac ischemia, is in stark contrast to the significant increases in myocardial lactate uptake (i.e., metabolic consumption) observed during exercise in lean animals. Changes in coronary venous O₂ saturation were consistent with changes in coronary venous PO₂ (Table 2). Neither arterial nor venous pH was changed in MetS swine compared with their respective lean controls at rest or during exercise (Table 2).

Figure 4 shows representative two-dimensional guided M-mode echocardiograms through the midleft ventricular level in lean and MetS swine. No statistically significant differences in cardiac dimensions, fractional shortening, stroke volume, or cardiac output were noted between conscious lean and MetS swine at rest (Table 3). However, cardiac index (normalization of cardiac output to body wt) was ∼25% lower in MetS vs. lean swine (P = 0.09).

Role of BKCa channels in local metabolic coronary vasodilation. Inhibition of BKCa channels with penitrem A (10 μg/kg iv) did not significantly affect blood pressure, heart rate, coronary blood flow, or MV̇O₂ in lean or MetS swine at rest or at the highest level of exercise (Table 2). Penitrem A had no effect on arterial or venous pH in either group of swine. Coronary venous PO₂ was not changed by penitrem A administration. Resting coronary venous PO₂ and coronary venous O₂ saturation were both diminished in MetS vs. lean swine. BKCa channel inhibition with penitrem A significantly lowered coronary venous PO₂ and O₂ saturation in lean swine at higher levels of MV̇O₂, but not in MetS. Interestingly, coronary venous O₂ saturation was increased by penitrem A in lean swine under resting conditions. However, Fig. 5 demonstrates that the relationship between coronary venous PO₂ and MV̇O₂ was not significantly affected by inhibition of BKCa channels in either lean (Fig. 5A) or MetS (Fig. 5B) swine. In fact, administration of penitrem A tended to increase coronary venous PO₂ at a given level of MV̇O₂ (Table 2); however, this effect failed to reach statistical significance (P = 0.10). It is important to point out that we previously demonstrated that the intravenous dose of penitrem A used in this investigation (10 μg/kg iv) is effective in inhibiting coronary vasodilation to the BKCa channel agonist NS-1619 in anesthetized, lean Ossabaw swine (3). Therefore, these data indicate that BKCa channels are not required for local metabolic coronary vasodilation during exercise.

DISCUSSION

The present study was designed to examine the hypothesis that impaired functional expression of coronary microvascular BKCa channels (3, 38) significantly contributes to the imbalance between myocardial oxygen supply and demand in MetS. The rationale for this hypothesis was based on recent evidence.

Fig. 2. Effect of MetS on mean aortic pressure (A) and heart rate (B) at rest and during graded treadmill exercise. Mean aortic pressure was not different at rest but was elevated with exercise intensity in MetS relative to lean swine. No differences in heart rate were noted between lean and MetS swine at rest or during exercise.

Fig. 3. Effect of MetS on the relationship between myocardial oxygen consumption and coronary venous PO₂ (A) and myocardial lactate uptake (B). The relationship between coronary venous PO₂ and myocardial oxygen consumption revealed a parallel downward shift in coronary venous PO₂ at a given level of myocardial oxygen consumption in MetS swine. This impairment of myocardial oxygen supply-demand balance was accompanied by a marked release of lactate (negative lactate uptake) in MetS swine as myocardial oxygen consumption was elevated.
The fact that the coronary venous PO2 vs. MV˙O2 relationship reserve in efforts to meet the tissue requirements for oxygen. Myocardium was forced to utilize its limited oxygen extraction in MetS vs. lean swine (Fig. 3). In other words, MetS expression of BKCa channels does not contribute to the impairment of myocardial metabolism [the primary determinant of coronary flow and/or myocardium do not regulate local metabolic control of coronary blood flow through a BKCa channel-dependent mechanism under physiological (lean) or pathophysiological (MetS) conditions. These data indicate that diminished functional expression of BKCa channels does not contribute to the impairment of myocardial oxygen-supply demand balance in MetS.

MetS and the control of coronary blood flow. Consistent with earlier studies (7, 29, 46, 54), data from this investigation further demonstrate that MetS significantly impairs the ability of the coronary circulation to adequately balance myocardial oxygen delivery with myocardial metabolism at rest and during exercise-induced increases in MV O2. This supply-demand imbalance is directly evidenced by the significant parallel downward shift in the relationship between coronary venous PO2 vs. MV O2 in MetS vs. lean swine (Fig. 3). In other words, MetS myocardium was forced to utilize its limited oxygen extraction reserve in efforts to meet the tissue requirements for oxygen. The fact that the coronary venous PO2 vs. MV O2 relationship shifted in a parallel manner indicates that MetS diminishes the balance between coronary blood flow and myocardial metabolism to an equal extent at rest and during exercise-induced increases in cardiac metabolism, i.e., increase in tonic constrictor and/or loss of a tonic dilator influence. Importantly, these data are consistent with our earlier study in MetS dogs (46). It is critical to recognize that simple examination of the coronary blood flow data in Table 2 do not adequately reflect the findings of the investigation, since they do not take into account the complex interaction between coronary blood flow and myocardial metabolism [the primary determinant of coronary flow (49)] or the various factors that influence perfusion and/or metabolism.

The impaired oxygen supply-demand balance in MetS is not associated with overt cardiac contractile dysfunction at rest, although there was a trend for lower cardiac index (cardiac output normalized to body wt) in MetS swine (Table 3 and Fig. 4), which is consistent with previous data in Yucatan swine (31) and the presence of “hyperdynamic circulation” (29). The marked increase in lactate release (negative lactate uptake) at higher levels of MV O2 indicates that the increase in oxygen extraction was inadequate, since myocardial underperfusion/ischemia was evidenced by the onset of anaerobic glycolytic metabolism (Fig. 3B). These data agree with earlier findings from our laboratory which documented that cardiac index is depressed at high levels of MV O2 in dogs with MetS (10). Furthermore, they suggest that the imbalance between coronary flow and metabolism positions MetS myocardium on the “brink” of ischemia at rest, such that exercise-induced increases in myocardial metabolism result in the onset of “demand ischemia.” Why myocardial oxygen extraction is maintained under these conditions (no decrease in coronary venous PO2) is intriguing, since an increase in oxygen utilization would act to mitigate the extent of hypoperfusion.

Although it can be argued that the elevated levels of insulin increased glucose uptake in MetS swine, thereby causing a switch in preferred substrate utilization of the heart, we propose that this is unlikely given that MetS swine are significantly insulin resistant, as evidenced by the elevation of both insulin and glucose in MetS swine (Table 1). The increase in cardiac lactate production is more indicative of elevated anaerobic glycolytic flux secondary to the impaired myocardial oxygen supply-demand balance, not a preferential “switch” in substrate utilization per se. Importantly, these changes are not related to the presence of a significant flow-limiting stenosis, since MetS Ossabaw swine exhibited only ~13% luminal narrowing of the coronary circulation (Fig. 1). It is possible that the echocardiograms missed microinfarcts that were present due to the myocardial ischemia present at all MV O2 levels, including near resting MV O2 (Figs. 3 and 4). Taken together, these data suggest that mechanisms that contribute to the imbalance between coronary blood flow and myocardial metabolism at rest and more importantly, during increases in MV O2, could underlie, at least in part, the increased incidence of myocardial ischemia, infarction, and sudden cardiac death observed in obese patients with MetS (26, 32). Microvascular dysfunction is likely to be a major contributing factor to overt cardiac dysfunction as MetS progresses to type 2 diabetes with gross hyperglycemia (31). Therefore, understanding the mechanisms responsible for the impairment of coronary flow regulation in MetS is critical to the treatment and possible prevention of these complications.

<table>
<thead>
<tr>
<th>Echocardiography data from lean and metabolic syndrome Ossabaw swine</th>
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<tbody>
<tr>
<td>Lean</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>End diastolic diameter, cm</td>
</tr>
<tr>
<td>End systolic diameter, cm</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
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<tr>
<td>Cardiac index, ml·min⁻¹·kg⁻¹</td>
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Values are means ± SE for lean (n = 4) and MetS (n = 4) swine.
BK$_{Ca}$ channels and local metabolic control of coronary blood flow. Given the abundant expression of BK$_{Ca}$ channels in coronary vascular smooth muscle cells (4, 22, 23, 38, 39) and the importance of K$^+$ channels to the regulation of coronary vascular resistance (15, 17, 53), we hypothesized that decreases in BK$_{Ca}$ channel function would likely contribute to diminished local metabolic coronary vasodilation in MetS. Our hypothesis is supported by a recent study from Merkus et al. (34) who found that administration of the BK$_{Ca}$ channel inhibitor tetraethylammonium (TEA) significantly decreased the relationship between coronary venous PO$_2$ and MV$_{O2}$ in normal, lean swine both at rest and during exercise. In addition, BK$_{Ca}$ channels have also been shown to contribute to the regulation of coronary microvascular tone in ischemic dog hearts (40). However, in contrast to our hypothesis, we found that the impaired balance between myocardial oxygen delivery and MV$_{O2}$ in MetS was not related to the diminished contribution of BK$_{Ca}$ channels to local metabolic control of coronary blood flow. In fact, our data fail to support a functional role for BK$_{Ca}$ channels in the regulation of coronary microvascular tone at rest or during exercise, since the inhibition of BK$_{Ca}$ channels with penitrem A did not significantly decrease the relationship between coronary venous PO$_2$ and MV$_{O2}$ in lean (Fig. 5A) or MetS (Fig. 5B) swine. Although this finding is directly at odds with the earlier study of Merkus et al. (34), it is important to recognize that TEA is also an autonomic ganglionic blocker that inhibits sympathetic output (12, 42). Therefore, TEA is a nonselective inhibitor that would not only affect the balance between coronary blood flow and MV$_{O2}$ by inhibiting BK$_{Ca}$ channels but also by decreasing $\beta$-adrenoceptor-mediated coronary vasodilation, a prominent, well-accepted mechanism of exercise-induced coronary vasodilation (14, 20, 24, 25, 37).

Even though BK$_{Ca}$ channels are known to contribute to adenosine-induced and endothelial [nitric oxide (NO)]-mediated dilation (3, 27, 35, 36), the lack of an effect of BK$_{Ca}$ channel inhibition on the balance between coronary blood flow and myocardial metabolism is not surprising, since numerous earlier studies have failed to establish a prominent role for adenosine or NO in exercise-induced coronary vasodilation (1, 2, 16, 28, 47, 50–52). Furthermore, we are confident that penitrem A effectively inhibits BK$_{Ca}$ channels, since we recently demonstrated that the intravenous dose of penitrem A used in this study significantly impairs coronary vasodilation in response to the BK$_{Ca}$ channel agonist NS-1619 in anesthetized, lean Ossabaw swine, and penitrem A is as effective as iberiotoxin in blocking BK$_{Ca}$ current in direct patch-clamp studies (3). We acknowledge that our experiments do not rule out possible compensatory activation of other K$^+$ channels that may have masked the role of BK$_{Ca}$ channels in local metabolic control of coronary blood flow at rest or during exercise-induced increases in MV$_{O2}$. Whether increases in the activity of other K$^+$ channels [i.e., ATP-dependent K$^+$ (K$_{ATP}$) and/or voltage-gated K$^+$ (Kv) channels] compensate to regulate coronary flow when BK$_{Ca}$ channels are inhibited is unknown and merits further study. However, it is important to recognize that numerous earlier studies have demonstrated that blockade of a single K$^+$ channel (K$_{ATP}$ or Kv) markedly reduces coronary flow and/or coronary venous PO$_2$; i.e., inhibition of either K$_{ATP}$ or Kv channels significantly reduces the balance between coronary flow and metabolism (8, 15, 17). We propose that if BK$_{Ca}$ channels were significantly contributing to the regulation of coronary vasomotor tone at rest or during exercise, then significant changes in flow and/or coronary venous PO$_2$ would be observed. Therefore, while our data do not rule out compensatory activation of other K$^+$ channels, they importantly demonstrate that BK$_{Ca}$ channels are not required in local metabolic control of coronary blood flow at rest or during exercise-induced increases in MV$_{O2}$.

In summary, our data indicate that factors released from the vascular endothelium and/or myocardium do not regulate local metabolic control of coronary blood flow through a BK$_{Ca}$ channel-dependent mechanism under physiological (lean) or pathophysiological (MetS) conditions. Thus diminished function of BK$_{Ca}$ channels (3, 6, 9, 33, 38) does not significantly contribute to the impairment of myocardial oxygen-supply demand balance at rest or during increases in MV$_{O2}$ in MetS. However, our findings do not contradict a role for decreases in BK$_{Ca}$ channel function contributing to coronary endothelial dysfunction that is typically observed in obesity, insulin resistance, and MetS (5, 29).

**DISCLOSURES**

No conflicts of interest are declared by the authors.

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