Impaired contractile recovery after low-flow myocardial ischemia in a porcine model of metabolic syndrome

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Clinical metabolic syndrome conveys a poor prognosis in patients with acute coronary syndrome, not fully accounted for by the extent of coronary atherosclerosis. To explain this observation, we determined whether postischemic myocardial contractile function and metabolic function are impaired in a porcine dietary model of metabolic syndrome without atherosclerosis. Micropigs (n = 28) were assigned to a control diet (low fat, no added sugars) or an intervention diet (high saturated fat and simple sugars, no added cholesterol) for 7 mo. The intervention diet produced obesity, hypertension, dyslipidemia, and impaired glucose tolerance, but not atherosclerosis. Under open-chest, anesthetized conditions, pigs underwent 45 min of low-flow myocardial ischemia and 120 min of reperfusion. In both diet groups, contractile function was similar at baseline and declined similarly during ischemia. However, after 120 min of reperfusion, regional work recovered to 21 ± 12% of baseline in metabolic syndrome pigs compared with 61 ± 13% in control pigs (P = 0.01). Ischemia-reperfusion caused a progressive decline in mechanical/metabolic efficiency (regional work/O2 consumption) in myocardial syndrome hearts, but not in control hearts. Metabolic syndrome hearts demonstrated altered fatty acyl composition of cardiolipin and increased Akt phosphorylation in both ischemic and nonischemic regions, suggesting tonic activation. Metabolic syndrome hearts used more fatty acid than control hearts (P = 0.03). When fatty acid availability was restricted by prior insulin exposure, differences between groups in postischemic contractile recovery and mechanical/metabolic efficiency were eliminated. In conclusion, pigs with characteristics of metabolic syndrome demonstrate impaired contractile and metabolic recovery after low-flow myocardial ischemia. Contributory mechanisms may include remodeling of cardiolipin, abnormal activation of Akt, and excessive utilization of fatty acid substrates.

metabolic syndrome; heart; ischemia; reperfusion; contractility; fatty acids; Akt; cardiolipin

“METABOLIC SYNDROME,” defined by the presence of a combination of obesity, hypertension, dyslipidemia, and/or diabetes and characterized by underlying insulin resistance, is a prevalent and growing problem in many populations. Recent United States data have indicated that 34% of all adults and 30% of nondiabetic adults fulfill criteria for the diagnosis of metabolic syndrome. Even in the absence of diabetes, the presence of metabolic syndrome predicts higher morbidity and mortality after acute coronary syndrome. This poor prognosis is not fully explained by the extent of coronary atherosclerosis, raising the question of whether abnormalities of myocardial metabolism are culpable. In most, but not all (24, 54, 59) rodent models of insulin resistance without diabetes, contractile recovery after myocardial ischemia is impaired compared with lean, insulin-sensitive controls. However, it is uncertain whether insulin resistance is associated with similar, adverse effects on postischemic contractile function of the human heart.

To bridge the gap between rodent and human pathophysiology, we have developed a porcine model that recapitulates many of the characteristics of clinical metabolic syndrome without diabetes. After 6 mo of diet enriched in saturated fat and simple sugars, pigs develop obesity, hypertension, dyslipidemia, and systemic and myocardial insulin resistance, but not diabetes. Because the intervention diet contains no cholesterol, atherosclerosis does not develop, allowing the effects of metabolic syndrome on cardiac function to be examined independently. In the present study, we used this model to determine whether metabolic syndrome impairs postischemic myocardial contractile function and investigated the mechanisms potentially responsible for such an effect.

Free fatty acids (FFAs) are the predominant energy substrates in the normal heart in vivo; however, oxidation of FFAs is impaired during ischemia, and toxic lipid intermediates may accumulate in the myocardium. In insulin-resistant states, myocardial substrate preference shifts toward greater utilization of FFAs and lower utilization of carbohydrate substrates, potentially exacerbating these effects. Accordingly, we hypothesized that excess availability and utilization of FFAs contributes to impaired recovery from myocardial ischemia in porcine metabolic syndrome.

Because metabolic syndrome may entail multiple abnormalities of energy metabolism and cell signaling, it is unlikely that any single mechanism would fully account for an effect of metabolic syndrome on responses to myocardial ischemia. Therefore, we investigated several other plausible mechanisms.
for such an interaction, including activities of pyruvate dehydrogenase (PDH) and AMP-activated protein kinase (AMPK), mitochondrial respiratory function, composition of cardiolipin, and activation of stress kinases.

MATERIALS AND METHODS

Animal model and dietary intervention. All experiments were approved by the Institutional Animal Care and Use Committee. Twenty-eight Yucatan micropigs (S&S Farms, Ramona, CA) were obtained at 3 mo of age and at baseline weighed 16 ± 1 kg. Some data from the pigs have been previously reported (27, 58). After a 2-wk period of acclimatization to the laboratory environment, pigs were assigned to either a control diet or an intervention diet with equal numbers of females and castrated males in each group. Details of the two diets have been previously published (27). The control diet was a high-fiber, low-fat laboratory minipig chow. The intervention diet used a base of standard minipig chow to which coconut oil [25% (wt/wt)] and high-fructose corn syrup [20% (wt/wt)] were added. The intervention diet provided excess fat, simple sugars, and calories, but both diet regimens provided nearly equal quantities of protein, essential fatty acids, and minerals. Neither diet was supplemented with cholesterol, thus avoiding the confounding influence of atherosclerosis (58).

Intravenous glucose tolerance testing. Study procedures are shown in Fig. 1. At baseline and after 6 mo of assigned diet, fasting blood samples were obtained, and the intravenous glucose tolerance test (IVGTT) was performed under moderate sedation with ketamine (25 mg/kg im) followed by a continuous infusion of ketamine (15 mg·kg⁻¹·h⁻¹ iv) and midazolam (0.35 mg·kg⁻¹·h⁻¹ iv). Details of the technique for IVGTT have been previously described (27). After the injection of 50% glucose solution (0.3 g/kg iv over 5 min), blood samples were collected at frequent intervals for 100 min for measurements of blood glucose and plasma insulin concentrations. Areas under the curve for the transient glucose and insulin responses were computed.

Echocardiography. In eight pigs from each diet group, transthoracic echocardiography was performed after 6 mo of assigned diet under moderate sedation as described above. Imaging was performed with a General Electric RT6800 echocardiography system and 2.5-MHz phased array transducer. Short-axis parasternal views of the left ventricle (LV) were obtained in all pigs. M-mode measurements of end-diastolic and end-systolic chamber diameter and posterior free wall thickness were obtained. With simultaneous systolic blood pressure measured in a leg with an automated sphygmomanometer (Omron), peak systolic wall stress and fractional systolic shortening were calculated using standard formulas (20).

Instrumentation of the heart for low-flow ischemia and reperfusion experiments. At 7 mo of assigned diet, pigs were sedated with ketamine (25 mg/kg im) and midazolam (0.5 mg/kg im), anesthetized with α-chloralose (100 mg/kg iv induction, 20–30 mg·kg⁻¹·h⁻¹ iv maintenance) and midazolam (0.35 mg·kg⁻¹·h⁻¹ iv), and wrapped in a recirculating warm water blanket to maintain body temperature. After endotracheal intubation, pigs were mechanically ventilated with O₂-enriched air; ventilation was adjusted based on measurements of arterial blood gases (1-Stat CG-4, Abbott, Princeton, NJ). Fluid-filled catheters were inserted in the jugular veins and a carotid artery for the administration of fluids and drugs, blood sampling, and measurement of aortic pressure. The chest was opened by median sternotomy, and the heart was suspended in a pericardial cradle. On the left atrial appendage, bipolar pacing electrodes were attached to maintain a constant heart rate slightly higher than the intrinsic rate, and a fluid-filled catheter was inserted for the injection of microspheres for blood flow measurement. To produce and monitor regional myocardial ischemia, an adjustable hydraulic occluder and a transit time ultrasonic flow probe (Transonic Systems, Ithaca, NY) were placed around the left anterior descending coronary artery (LAD) just distal to the first diagonal branch. To measure regional contractile function, a solid state micromanometer catheter was inserted in the LV, and an array of four piezoelectric transducers (two orthogonal pairs) was implanted in the subendocardium of the anterior LV free wall to measure regional wall area (i.e., a two-dimensional equivalent to segment length) by sonomicrometry (Sonometrics, London, ON, Canada). Hemodynamic data were digitized at 200 Hz and analyzed with custom software to plot LV pressure versus regional wall area. The area of each pressure-wall area loop reflects regional external work performed in one cardiac cycle.

Myocardial substrate uptake. To eliminate differences in arterial glucose concentration as an independent variable influencing myocardial substrate uptake, arterial blood glucose was clamped at 4.5–5.0 mmol/l in all pigs for the entire duration of experiments by an infusion of 20% glucose through a jugular vein at a rate determined by iterative arterial blood glucose sampling (27). This blood glucose level is intermediate between those measured under fasting conditions and at

![Fig. 1. Experimental design. Details of control and metabolic syndrome (Met Syn) diets are provided in Ref. 27. IVGTT, intravenous glucose tolerance test; BP, blood pressure; FFA, free fatty acids.](http://ajpheart.physiology.org/Content/dm/862/AJP-Heart-Circ-Physiol)
the peak response to IVGTG and thus is within a range likely achieved by the pigs with normal feeding.

Arteriovenous concentration differences of glucose, lactate, FFAs, O2, and CO2 were determined in duplicate under each experimental condition by paired sampling of arterial and anterior interventricular vein blood. In pigs, 90% of the blood flow of the anterior interventricular vein is derived from the perfusion territory of the LAD (5). Therefore, to sample coronary venous blood from the region to be rendered ischemic, a fluid-filled catheter was inserted in the anterior interventricular coronary vein at a point adjacent to the site of the occluder on the LAD. Blood glucose and lactate were measured with an auto-analyzer (2300 Stat, Yellow Springs Instruments, Yellow Springs, OH). FFAs were measured by a colorimetric assay (Wako Diagnostics, Richmond, VA). Hematocrit was used to convert plasma FFA concentration to blood concentration. Total O2 content was computed from the sum of dissolved O2 content (calculated from PO2) and hemoglobin-bound O2 content (OSM3, Radiometer, Copenhagen, Denmark). Total CO2 content and PCO2 were measured using an I-Stat CG-4 blood gas analyzer. Plasma insulin was measured using an automated sandwich ELISA method (Access Ultrasensitive Insulin; Beckman Coulter, Fullerton, CA).

Myocardial uptake or production of substrates, O2, or CO2 in the perfusion territory of the LAD was calculated as the product of tissue blood flow in the anterior LV free wall and the arteriovenous concentration difference (aorta – anterior interventricular vein). To measure tissue blood flow, 3.5 × 10−15 μm polyestrene fluorescent (Invitrogen, Carlsbad, CA) or stable isotope (42) microspheres were injected into the left atrium while a reference arterial blood sample was withdrawn from a carotid artery with a calibrated pump.

Ischemia-reperfusion protocols. All pigs underwent 45 min of low-flow myocardial ischemia (LAD blood flow reduced to 55% of baseline, ±1 ml/min) followed by 120 min of reperfusion. Measurements were made at the following four time points: baseline (preisch), 35–45 min of ischemia, 20–30 min of reperfusion, and 110–120 min of reperfusion. The primary outcome measure was regional contractile function (external work) at 110–120 min of reperfusion. We have previously demonstrated that ischemia of this severity, sustained for up to 90 min, results in stunning without infarction in pigs (46).

Sixteen pigs (8 pigs/diet group) were studied under conditions of ambient FFA availability (protocol A). Twelve additional pigs (6 pigs/diet group) were studied under conditions of restricted FFA availability (protocol B). This was achieved by exposing the pigs to hyperinsulinemic, euglycemic conditions (glucose: 4.5–5.0 mmol/l, insulin: 1–2 μU·kg−1·min−1 iv) for a 90-min period spanning the interval 180–90 min before the onset of ischemia. Plasma insulin concentration reached a mean of 175 μU/ml during this period. Insulin infusion was discontinued 90 min before ischemia, whereas glucose remained clamped at 4.5–5.0 mmol/l. The hiatus between insulin exposure and ischemia allowed plasma insulin concentration to return to baseline before ischemia. The hiatus between insulin exposure and ischemia allowed plasma insulin concentration to return to baseline before ischemia, and prior data indicated that this hiatus was sufficient to avoid persistent activation of myocardial phosphatidylinositol 3-kinase (PI3K) and Akt during reperfusion (27). However, the antilipolytic effect of insulin persisted throughout ischemia and reperfusion, as reflected by low arterial FFA concentrations. After the completion of in vivo measurements, pigs were euthanized, and myocardial tissue samples rapidly excised from the central portion of the ischemic region (anterior LV free wall) and from a remote, nonischemic region (posterolateral LV free wall). Samples were divided into subendocardial and subepicardial layers, weighed, and frozen at −80°C for subsequent biochemical and microsphere blood flow analysis.

Lactate oxidation. In protocol A pigs, myocardial lactate oxidation was measured by the transmyocardial production of 13CO2 from [1-13C]lactate (Isotec, St. Louis, MO) (21). After the metabolism of lactate to pyruvate by lactate dehydrogenase, CO2 is liberated from the 1-C position by the action of PDH. The bicarbonate pool was primed with [13C]NaHCO3 (0.12 mg/kg iv, Isotec), and a primed, continuous infusion of [1-13C]lactate (1.1 mg/kg prime, 0.033 mg·kg−1·min−1 iv) was initiated 2 h before the onset of ischemia. Arterial blood samples were obtained every 30 min for 120 min to document steady-state lactate enrichment. Lactate isotopic enrichment was determined from 100 μl of plasma and analyzed using the trimethylsilyl derivative of lactate on a Agilent 6890/5973 GC/MS using standard methods (57). Blood 13CO2 enrichment was measured using isotope ratio mass spectroscopy. At each experimental condition, myocardial lactate oxidation was calculated as follows (4, 21):

Myocardial lactate oxidation (in μmol·g−1·min−1) = (coronary venous − arterial)13CO2/arterial enrichment of lactate × myocardial blood flow rate

As an indicator of the nonaerobic metabolism of glucose, myocardial lactate release was calculated as follows:

Myocardial lactate release (in μmol·g−1·min−1) = (net chemical lactate extraction − [13C] tracer-measured lactate extraction) × myocardial blood flow rate

AMPK. In protocol A pigs, total and active forms of AMPK were assessed by Western blot analysis of subendocardial samples from ischemic-reperfused and nonischemic regions. Tissue was homogenized over ice using a cocktail of CytoBuster (Novagen Protein Extraction) with protease and phosphatase inhibitors, further processed with Invitrogen sample reducing agent, and heated at 70°C for 10 min. Aliquots of 12–15 μg were loaded onto precast minigels (Invitrogen). After transfer, membranes were blocked overnight using 5% nonfat milk in Tris-buffered saline with Tween 20 (TBST).

Membranes were washed with TBST for three 8-min cycles. Total AMPK was identified using a AMPK-α polyclonal rabbit primary antibody (sc-74461, Santa Cruz Biotechnology, Santa Cruz, CA; 1:600 in 3% BSA) and secondary horseradish peroxidase-linked donkey anti-rabbit antibody (1:3,300 in 3% BSA). Phosphorylation of AMPK α1- and α2-subunits at Thr172 was identified using polyclonal rabbit primary antibody (sc-33524, Santa Cruz Biotechnology; 1:300 in 3% BSA) with the same secondary antibody. The membrane was visualized using a chromogenic membrane development system (Millipore).

PDH activity and quantity and pyruvate dehydrogenase kinases. In protocol A pigs, homogenates were prepared from subendocardial samples of ischemic-reperfused and remote nonischemic myocardium. PDH content and activity were measured (Mitosciences, Eugene, OR). PDH from crude mitochondrial extracts was immunopurified in microplate wells using monoclonal antibody to the E2 subunit of the PDH complex, resulting in a system free of lactate dehydrogenase and other potential tissue contaminants. PDH quantity was measured by ELISA. PDH activity was determined by spectrophotometric analysis of the rate of NADH production after the addition of pyruvate substrate (30).

Contents of pyruvate dehydrogenase kinase (PDK)2 and PDK4, key regulators of PDH activity, were measured by Western blot analysis using rabbit monoclonal anti-PDK2 antibody (22828-1, Epitomics, Burlingame, CA) and mouse monoclonal anti-PDK4 antibody (MSP 52, Mitosciences) (both 1:1,000 in 2% BSA-TBST).

Secondary detection used horseradish peroxidase-linked donkey anti-rabbit or anti-mouse antibody (1:1,000 in 2% BSA-TBST).

Akt and ERK1/2. In protocol A pigs, Akt and ERK (ERK1/2 isoforms) were assessed in subendocardial samples from ischemic-reperfused and nonischemic regions by Western blot analysis. Total Akt and ERK1/2 were identified with primary rabbit polyclonal antibodies (Cell Signaling, Danvers, MA; 1:1,000 in 5% BSA) and secondary alkaline phosphatase-linked anti-rabbit antibody visualized using BCIP/NBT (NDS, Jackson, CA).

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with the CDP-Star development system (New England BioLabs, Ipswich, MA). Activated Akt and ERK were detected using monoclonal rabbit antibodies recognizing the phosphorylation of Akt at Ser473 and phosphorylation of ERK1/2 at Thr202 and Tyr204 (New England BioLabs; 1:1,000 in 5% BSA) and secondary alkaline phosphate-linked anti-mouse (for phospho-Akt) or anti-rabbit (for phospho-ERK) antibody visualized with CDP-Star.

Mitochondrial respiration and myocardial cardiolipin. In protocol A pigs, interstitial and subsarcolemmal mitochondria were isolated from fresh myocardial tissue samples. Briefly, mitochondria were freshly isolated from 500–750 mg of LV tissue in ice-cold Chappell-Perry buffer consisting of (in mM) 100 KCl, 50 MOPS, 1 EGTA, 5 MgSO4, 7H2O, and 1 ATP (pH 7.4 with KOH) by standard differential centrifugation methods as previously described (40). State 3 (ADP concentration) and state 4 (ADP limited) mitochondrial respiratory function was measured in isolated mitochondria (0.25 mg protein) using a Clark-type electrode system (Strathkelvin, North Lanarkshire, Scotland) at 30°C with pyruvate (5 mM) and malate (5 mM) as substrates to examine effects on carbohydrate versus lipid supported oxidation pathways (10). The respiratory control ratio (state 3/state 4) was also calculated. LV myocardial cardiolipin composition was determined in pigs from protocols A and B using electrospray ionization MS coupled to HPLC on an API 3200 LC/MS (AB Sciex, Framingham, MA), as previously described (50). Total cardiolipin was calculated using the sum of the eight most abundant molecular species.

Statistical approach. For measurements repeated over time or in two myocardial regions, differences between control and metabolic syndrome groups and the interaction of diet with time were determined by two-way repeated-measures ANOVA. Comparisons at individual time points were performed with unpaired t-tests.

RESULTS

Characteristics of metabolic syndrome. Before diet assignment, both groups had similar weight, blood pressure, blood lipids, and insulin sensitivity, as determined by IVGTT. After 6 mo, pigs fed a high-fat, high-sugar diet developed characteristics of classical metabolic syndrome, including obesity, elevated blood pressure, dyslipidemia, elevated fasting blood glucose and insulin levels, and insulin resistance based on the results of IVGTT (Tables 1 and 2). Pathological examination of the thoracic aorta and coronary arteries previously revealed no differences between groups in intima-media thickness and no histological evidence of atherosclerosis in either group (58). At euthanasia after 7 mo of assigned diet, heart weight was significantly greater, but heart weight/body weight was significantly lower, in pigs with characteristics of metabolic syndrome compared with control pigs.

Echocardiography. Findings on echocardiography performed after 6 mo of assigned diet under nonischemic conditions in protocol A are shown in Table 1. Fractional systolic shortening was normal in both groups and did not differ between groups. Pigs with characteristics of metabolic syndrome had significantly greater LV wall thickness; however, systolic wall stress was similar to the control group, indicating that LV hypertrophy appropriately compensated for increased systolic blood pressure.

Effects of low-flow ischemia and reperfusion on hemodynamics and contractile function. One pig in the metabolic syndrome group demonstrated no reactive hyperemia upon the release of coronary constriction and no significant ischemia.

Table 2. Glucose, insulin, and lipids during chronic dietary intervention

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>Met Syn Diet</th>
<th>P Value</th>
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<tbody>
<tr>
<td></td>
<td>0 mo</td>
<td>6 mo</td>
<td>0 mo</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/l</td>
<td>2.48 ± 0.10</td>
<td>2.32 ± 0.07</td>
<td>2.55 ± 0.12</td>
</tr>
<tr>
<td>Fasting plasma insulin, μU/ml</td>
<td>2.5 ± 0.4</td>
<td>2.4 ± 0.2</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>Intravenous glucose tolerance testing</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>AUC for glucose, mmol·1⁻¹·min⁻¹</td>
<td>376 ± 13</td>
<td>386 ± 23</td>
<td>384 ± 16</td>
</tr>
<tr>
<td>AUC for insulin, μU·ml⁻¹·min⁻¹</td>
<td>727 ± 68</td>
<td>978 ± 151</td>
<td>594 ± 79</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/l</td>
<td>1.94 ± 0.07</td>
<td>1.79 ± 0.07</td>
<td>1.81 ± 0.05</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/l</td>
<td>0.38 ± 0.05</td>
<td>0.27 ± 0.02</td>
<td>0.35 ± 0.03</td>
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</table>

Data are means ± SE; n = 14 pigs/group (protocol A and B pigs). AUC, area under the curve. *P < 0.01 for difference between groups at 6 mo; †P ≤ 0.01 for interaction of diet and time.
from microsphere blood flow measurements. Therefore, this pig was excluded from analyses of the effects of ischemia and reperfusion. Hemodynamic and myocardial blood flow data for protocol A are shown in Table 3. At baseline, there were no differences in these variables between diet groups. In particular, baseline (preischemic) contractile function did not differ between groups, as reflected by similar values of regional external work, myocardial blood flow, oxygen consumption, and contractile function were reduced to a similar extent in both groups (Fig. 2).

Pronounced differences in contractile and metabolic recovery emerged between groups during reperfusion. Control pigs exhibited a partial recovery of regional work, reaching 61 ± 13% of baseline at 120 min of reperfusion (Fig. 2A). In contrast, metabolic syndrome pigs exhibited a further decline in regional work during reperfusion, to 21 ± 12% of baseline at 120 min of reperfusion (P = 0.01 vs. control pigs). Myocardial O2 consumption (MV\textsuperscript{2}O\textsubscript{2}) remained nearly constant during reperfusion in control pigs and rose slightly in metabolic syndrome pigs (Fig. 2B). Mechanical/metabolic efficiency, defined as the quotient of regional work/MV\textsuperscript{2}O\textsubscript{2}, remained relatively constant throughout the experiment in control pigs, whereas metabolic syndrome pigs exhibited a marked and progressive decline in this index (Fig. 2C).

Effects of low-flow ischemia and reperfusion on energy substrate metabolism and mitochondrial respiration. Metabolic syndrome hearts had greater FFA extraction and uptake than control hearts (both P = 0.03 for effect of diet over the course of the protocol; Table 4 and Fig. 3A). Differences were most pronounced at baseline and during early reperfusion. Despite higher mean plasma insulin concentrations, metabolic syndrome hearts tended to have lower glucose uptake (P = 0.05 vs. control pigs). Myocardial O2 consumption (MV\textsuperscript{2}O\textsubscript{2}) remained relatively constant throughout the experiment in control pigs and rose slightly in metabolic syndrome pigs (Fig. 2B). Mechanical/metabolic efficiency, defined as the quotient of regional work/MV\textsuperscript{2}O\textsubscript{2}, remained relatively constant throughout the experiment in control pigs, whereas metabolic syndrome pigs exhibited a marked and progressive decline in this index (Fig. 2C).

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0.12 for effect of diet over the course of the protocol; Fig. 3B). Mean arterial lactate concentration was within the range of 0.9–1.1 mmol/l in both groups under all experimental conditions. There were no differences between groups in either lactate oxidation or lactate release as determined by $^{13}$C isotopomer techniques (Fig. 4, A and B).

Coronary venous pH did not differ between groups under baseline conditions (control group: 7.32 ± 0.02 and metabolic syndrome group: 7.38 ± 0.02) and declined to a similar extent during ischemia (control group: 7.23 ± 0.02 and metabolic syndrome group: 7.27 ± 0.02). However, the transmyocardial CO$_2$ gradient (MV$_{CO_2}$) during ischemia was significantly greater in metabolic syndrome hearts than in control hearts ($P = 0.02$; Fig. 4C).

Mitochondrial respiration rates in the nonischemic myocardium were similar in control and metabolic syndrome hearts. In interﬁbrillar mitochondria, ischemia-reperfusion tended to depress state 3 respiration in metabolic syndrome hearts but not in control hearts ($P = 0.15$ for interaction of diet group and ischemia on state 3 respiration; Fig. 5). Ischemia-reperfusion had opposite effects on the respiratory control ratio in control versus metabolic syndrome hearts: in control hearts, the respiratory control ratio with malate/pyruvate or succinate substrate tended to be higher in the ischemic-reperfused myocardium than in the nonischemic myocardium; in metabolic syndrome hearts, the respiratory control ratio with these substrates tended to be lower in the ischemic-reperfused myocardium than in the nonischemic myocardium (data not shown). There was significant interaction of diet and region on the respiratory control ratio ($P = 0.02$). Respiration of subsarcolemmal mitochondria did not differ between groups (data not shown).

**AMPK, PDH, PDK2, and PDK4.** There were no differences between diet groups or between ischemic and nonischemic regions in the expression of total or phosphorylated AMPK, quantity or activity of PDH, or expression of PDK2 or PDK4 (Table 5).

**ERK1/2 and Akt phosphorylation.** Total (nonphosphorylated and phosphorylated) ERK1/2 and Akt did not differ between diet groups or myocardial regions. Ischemia caused significant phosphorylation of ERK1/2 in both diet groups ($P = 0.003$ for main effect of ischemia; Fig. 6A) and modest phosphorylation of Akt at Ser473 ($P = 0.09$ for main effect of ischemia; Fig. 6B) without a significant interaction of diet and ischemia on phos-
phorylation of either kinase. However, phosphorylation of Akt at Ser473 was greater in both nonischemic and ischemic-reperfused regions of metabolic syndrome hearts compared with the corresponding regions of control hearts (P < 0.04 for main effect of diet). This finding suggests a tonic overactivation of Akt in the myocardium of metabolic syndrome hearts.

Cardiolipin composition. Total cardiolipin content did not significantly differ by diet or by myocardial region (Table 6). Content of (18:2)₄-cardiolipin represented ~70% of total cardiolipin and also showed no differences between diets or myocardial regions. However, metabolic syndrome hearts had increased content of several cardiolipin species in which shorter-chain fatty acid moieties were substituted for linoleic acid. These included cardiolipin with one or two myristic acid (14:0) residues or one palmitoleic (16:1) residue. Concentrations of these cardiolipin species were significantly lower in ischemic-reperfused regions compared with nonischemic regions. Two species of cardiolipin with unsaturated oleic (18:1) and arachidonic (20:4) residues were diminished in metabolic syndrome hearts.

Effect of prior insulin exposure, resulting in restricted FFA availability. In protocol B pigs, prior exposure to hyperinsulinemic euglycemic conditions resulted in low arterial FFA concentrations in both diet groups during the ischemia-reperfusion experiment (Table 7) compared with FFA concentrations in pigs studied under protocol A (ambient FFA availability; Table 4). However, arterial glucose, lactate, and insulin concentrations during ischemia and reperfusion were similar in protocols A and B in both diet groups.

As expected, the reduced FFA availability in protocol B altered the pattern of myocardial substrate uptake. Myocardial FFA uptake was negligible in both groups and did not differ between groups (Fig. 7A), whereas myocardial glucose uptake was greater than in protocol A in both diet groups (Fig. 7B).

Before ischemia in protocol B, regional work was 20 ± 2 cm²·mmHg in control pigs and 18 ± 2 cm²·mmHg in metabolic syndrome pigs, levels similar to protocol A. With ischemia in protocol B, regional work declined to a similar extent in both diet groups and to a similar extent as in protocol A. However, after reperfusion in protocol B, contractile recovery was substantially greater than in protocol A (Fig. 7C compared with Fig. 2B), particularly among metabolic syndrome pigs. Differences between diet groups in regional work or mechanical/metabolic efficiency (Fig. 7D) were eliminated in protocol B.
In a porcine dietary model that recapitulates many clinical features of metabolic syndrome, this study shows that metabolic syndrome hearts, compared with normal hearts, are poised for profound contractile dysfunction and reduced mechanical/metabolic efficiency after relatively brief low-flow ischemia. Because metabolic syndrome involves complex derangements of cell metabolism and signaling, it is not surprising that multiple mechanisms may contribute to poor ischemic tolerance. In this study, excess myocardial fatty acid utilization, altered cardiolipin composition, and abnormal activation of Akt were identified as potential contributory mechanisms. Prior insulin exposure, resulting in restricted FFA availability, eliminated differences between diet groups in postischemic contractile function and mechanical/metabolic efficiency.

An important difference between the present and prior porcine dietary models of metabolic syndrome is that prior models have used diets supplemented with cholesterol, predisposing to the development of atherosclerosis (16, 26). In contrast, the intervention diet in the present model contained no added cholesterol, thereby avoiding the development of atherosclerosis (58) and allowing the effects of metabolic syndrome on the myocardium to be evaluated independently.

**Hemodynamics and myocardial blood flow.** Although systolic blood pressure under sedated conditions was greater in metabolic syndrome pigs than in control pigs, there were no differences between groups in arterial blood pressure, heart rate, or myocardial blood at any time point of the terminal ischemia-reperfusion experiment under general anesthesia. Therefore, differences in contractile and metabolic recovery after ischemia are unlikely to be due to differences in hemodynamics or perfusion.

**Myocardial glucose and fatty acid utilization.** The results from protocol A demonstrated a shift in substrate utilization in metabolic syndrome hearts from glucose to FFAs. The results from protocol B suggest that excessive utilization of FFA may contribute to poor ischemic tolerance in metabolic syndrome hearts.

**Glucose protects the heart in ischemia and reperfusion** (14); conversely, animal and human data have indicated that systemic insulin resistance is accompanied by myocardial insulin resistance and impaired glucose utilization (7, 43, 61). We (27) previously observed defective myocardial insulin signaling and blunted myocardial glucose uptake in response to exogenous insulin in our porcine model of metabolic syndrome. In this study, despite significantly higher plasma insulin concentrations, myocardial glucose uptake tended to be lower in the metabolic syndrome group than in the control group (Fig. 3B), consistent with myocardial insulin resistance in the former. Neither myocardial lactate oxidation nor lactate release differed between groups, indicating that there were no differences between diets or regions for any of the analytes.

**Table 5. Myocardial PDH, PDK2, PDK4, and AMPK**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Control Diet Nonischemic region</th>
<th>Ischemic-reperfused region</th>
<th>Met Syn Diet Nonischemic region</th>
<th>Ischemic-reperfused region</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDH activity</td>
<td>1.00 ± 0.09</td>
<td>0.88 ± 0.06</td>
<td>0.82 ± 0.06</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>PDH specific activity</td>
<td>1.00 ± 0.10</td>
<td>1.03 ± 0.05</td>
<td>0.96 ± 0.05</td>
<td>1.03 ± 0.06</td>
</tr>
<tr>
<td>PDK2 expression</td>
<td>1.00 ± 0.05</td>
<td>1.07 ± 0.02</td>
<td>1.02 ± 0.03</td>
<td>1.07 ± 0.04</td>
</tr>
<tr>
<td>PDK4 expression</td>
<td>1.00 ± 0.03</td>
<td>1.08 ± 0.04</td>
<td>1.00 ± 0.02</td>
<td>0.99 ± 0.03</td>
</tr>
<tr>
<td>Phosphorylated/total AMPK</td>
<td>1.00 ± 0.08</td>
<td>1.01 ± 0.06</td>
<td>1.04 ± 0.09</td>
<td>1.10 ± 0.08</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 7–8 pigs/diet for each region (protocol A pigs). PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; AMPK, AMP-activated protein kinase. Data were normalized to the mean value in the nonischemic region of control diet group. There were no significant differences between diets or regions for any of the analytes.
between groups in the rates of oxidative metabolism of lactate through PDH or of nonaerobic glycolysis, respectively.

The greater dependence on FFAs as energy substrates, compared with control hearts, may have contributed to impaired contractile and metabolic recovery after ischemia in metabolic syndrome hearts. Before ischemia, contractile function of metabolic syndrome hearts was normal despite a shift toward FFAs as energy substrates. However, conditions of limited myocardial O\textsubscript{2} availability during ischemia may result in incomplete FFA oxidation and the accumulation of neutral lipid, FFA, and fatty acyl metabolites, an effect that is magnified by higher arterial FFA concentrations (2, 62). Among these metabolites, long-chain acyl-CoA and carnitine may be toxic to mitochondria, decreasing the efficiency of oxidative phosphorylation (31). Drugs that limit the accumulation of fatty acyl intermediates during ischemia improve contractile recovery, whereas drugs that increase the accumulation of those intermediates exacerbate contractile dysfunction (34).

Saturated fatty acids, in particular, exacerbate lipid toxicity and metabolic inefficiency during ischemia and reperfusion (41). For example, unoxidized palmitoyl-CoA may condense with serine to form ceramide, an initiator of apoptosis (18) and an intermediate known to promote inflammation and insulin resistance (52). The dietary fat supplement in our model, coconut oil, is among the most highly saturated natural oils, with \approx 90% saturated moieties, particularly lauric (12:0), myristic (14:0), and palmitic (16:0) acids. We (27) have previously demonstrated that this diet alters the composition of myocardial lipid pools, with increases in 12:0, 14:0, and 16:0 saturated fatty acyl residues and decreases in polyunsaturated 18:2 (linoleic) residues. Myristic acid may exert specific effects to promote the formation of ceramide by myrissoylation of the enzyme dihydroceramide Δ4-desaturase (3).

To explore whether greater availability and utilization of FFAs contributed to poorer contractile and metabolic recovery and impaired mechanical/metabolic efficiency in metabolic syndrome hearts, we conducted protocol B, using a period of euglycemic hyperinsulinemia before ischemia. The antilipolytic effects of insulin persisted during ischemia and reperfusion, resulting in low arterial FFA concentrations and myocardial FFA uptake. However, the delay of 90 min from the end of hyperinsulinemia to the onset of ischemia allowed plasma insulin concentration to return to baseline, and in previous experiments avoided persistent insulin signaling through PI3K and Akt (27). Under these conditions of restricted FFA availability, posts ischemic contractile recovery and mechanical/metabolic efficiency were improved in metabolic syndrome hearts and no longer differed from the measures in control hearts. It is unlikely that myocardial glycogen loading accounts for the benefit of insulin before ischemia in metabolic syndrome hearts, because net chemical lactate release by metabolic syndrome hearts during ischemia was no greater in protocol B (0.27 \pm 0.08 \text{μmol·g}^{-1}·\text{min}^{-1}) than in protocol A (0.36 \pm 0.06 \text{μmol·g}^{-1}·\text{min}^{-1}). However, we cannot exclude the possibility that other unmeasured, persistent effects of preceding insulin treatment contributed to the improved ischemic tolerance of metabolic syndrome hearts in protocol B.

**AMPK and PDH.** Reduced activities of AMPK and PDH have been observed in the myocardium in models of insulin resistance and/or high-fat feeding (1, 9, 25, 51), and reduced activity of either enzyme exacerbates myocardial contractile dysfunction after ischemia and reperfusion (29, 44). However, in the present model, we found no evidence that either assigned

### Table 6. Myocardial cardiolipin composition

<table>
<thead>
<tr>
<th>Species with increased concentration in the Met Syn group</th>
<th>Control Diet</th>
<th>Met Syn Group</th>
<th>Nonischemic region</th>
<th>Ischemic-reperfused region</th>
<th>Nonischemic region</th>
<th>Ischemic-reperfused region</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,448 (18:2)\textsubscript{x}</td>
<td></td>
<td></td>
<td>28.1 ± 1.6</td>
<td>30.5 ± 2.6</td>
<td>27.6 ± 1.6</td>
<td>25.4 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>1,422 (16:1) + (18:2)\textsubscript{v}</td>
<td>1.64 ± 0.33</td>
<td>1.24 ± 0.23</td>
<td>5.19 ± 1.05</td>
<td>4.05 ± 0.92</td>
<td>§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,396 (14:0) + (18:2)\textsubscript{x}</td>
<td>0.22 ± 0.05</td>
<td>0.17 ± 0.08</td>
<td>1.08 ± 0.06</td>
<td>0.80 ± 0.07</td>
<td>*§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,368 (14:0)\textsubscript{y} + (18:2)\textsubscript{z}</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.83 ± 0.11</td>
<td>0.46 ± 0.07</td>
<td>*§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species with decreased concentration in the Met Syn group</td>
<td></td>
<td></td>
<td>4.6 ± 30.5</td>
<td>2.3 ± 43.4</td>
<td>2.2 ± 37.4</td>
<td>3.0 ± 37.4</td>
<td>NS</td>
</tr>
<tr>
<td>1,476 (18:1)\textsubscript{y} + (18:2) + (20:4)</td>
<td>2.48 ± 0.16</td>
<td>2.72 ± 0.22</td>
<td>1.25 ± 0.15</td>
<td>1.49 ± 0.12</td>
<td>†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,474 (18:1) + (18:2) + (20:4)</td>
<td>0.75 ± 0.13</td>
<td>0.92 ± 0.15</td>
<td>0.57 ± 0.11</td>
<td>0.51 ± 0.10</td>
<td>†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cardiolipin</td>
<td>40.0 ± 3.3</td>
<td>43.4 ± 3.3</td>
<td>40.8 ± 2.2</td>
<td>37.4 ± 3.0</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE (in nmol/mg tissue protein); n = 14–16 pigs/group for each region (protocol A and B pigs). *P < 0.05 for main effect of diet; †P < 0.05 vs. the control group. ‡P < 0.05 for main effect of ischemia-reperfusion; §P < 0.05 for interaction of diet and ischemia by two-way repeated-measures ANOVA.

### Table 7. Arterial substrate and insulin concentrations during ischemia-reperfusion with restricted FFA availability

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control Diet</th>
<th>Met Syn Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.6 ± 0.1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Ischemia (90 min)</td>
<td>4.6 ± 0.1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Reperfusion (120 min)</td>
<td>4.7 ± 0.1</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>Plasma FFA, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.07 ± 0.02</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>Ischemia (90 min)</td>
<td>0.10 ± 0.02</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>Reperfusion (120 min)</td>
<td>0.11 ± 0.03</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>Blood lactate, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Ischemia (90 min)</td>
<td>1.3 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Reperfusion (120 min)</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3 ± 0</td>
<td>18 ± 2*</td>
</tr>
<tr>
<td>Ischemia (90 min)</td>
<td>3 ± 0</td>
<td>18 ± 2*</td>
</tr>
<tr>
<td>Reperfusion (120 min)</td>
<td>5 ± 1</td>
<td>17 ± 3*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6 pigs/group (protocol B pigs). *P < 0.05 vs. the control group.
diet or ischemia-reperfusion influenced the phosphorylation of AMPK, PDH activity, or expression of the major PDH regulatory kinases PDK2 and PDK4 in the myocardium. Therefore, abnormalities of these enzymes are unlikely to account for the central finding of impaired ischemic tolerance in metabolic syndrome hearts.

During ischemia, MV˙CO2 was greater in metabolic syndrome hearts than in control hearts (Fig. 4C). The primary mechanisms for transmyocardial CO2 production are the formation of CO2 as the end product of oxidative substrate metabolism and the formation of CO2 via the carbonic anhydrase reaction, when protons from lactic acid or ATP hydrolysis are buffered by tissue and blood bicarbonate (13). MV˙O2 and myocardial substrate uptake during ischemia were similar in both groups; therefore, the difference in MV˙CO2 is unlikely to be due to a difference of oxidative substrate metabolism. Lactate release did not differ between groups (Fig. 4B), and therefore a difference in proton generation from lactic acid is unlikely to explain the difference in MV˙CO2. Rather, the greater MV˙CO2 in metabolic syndrome hearts during ischemia may reflect greater net hydrolysis of high-energy phosphates, an effect that could contribute to postischemic contractile dysfunction.

Mitochondrial function and cardiolipin. In vivo mechanical/metabolic efficiency declined with ischemia and reperfusion in metabolic syndrome hearts but not in control hearts (Fig. 2C). In vitro, state 3 respiration of interfibrillar mitochondria from the ischemic-reperfused region tended to be reduced compared with mitochondria from the nonischemic region in metabolic syndrome hearts, but not in control hearts (Fig. 5). Although the interaction of diet and ischemia on state 3 respiration was not statistically significant, there was significant interaction on the respiratory control ratio. In summary, the in vivo and in vitro findings suggest that ischemia-reperfusion may have provoked greater energetic dysfunction in metabolic syndrome hearts compared with control hearts.

Cardiolipin is a phospholipid component of the inner mitochondrial membrane that is essential for normal mitochondrial electron transport. Cardiac cardiolipin is primarily composed of molecules that contain four linoleic acid (18:2) moieties; however, the composition of cardiolipin can be influenced by the composition of dietary fat (23), with substitution of other fatty acyl moieties for linoleic acid and consequent compromise of mitochondrial function (49).

In the present experiments, myocardial cardiolipin in metabolic syndrome pigs contained excess short-chain fatty acids, presumably reflecting dietary availability. The concentrations of cardiolipin species with short-chain fatty acyl residues were significantly lower in the ischemic-reperfused myocardium compared with the nonischemic myocardium, whereas other species of cardiolipin were unaffected by ischemia-reperfusion. These findings may indicate that cardiolipin with short-chain fatty acyl residues is particularly susceptible to degradation by phospholipases that are activated by ischemia. Since cardiolipin in its normal acyl form (containing linoleic acid residues) is essential for the function of many mitochondrial proteins, including cytochrome c (11), any disruption in its acyl composition may have detrimental effects on mitochondrial function. In summary, metabolic syndrome pigs demonstrated pathological remodeling of myocardial cardiolipin that may
have predisposed the heart to mitochondrial dysfunction and metabolic inefficiency after ischemia.

**Stress kinase activation.** During myocardial ischemia and reperfusion, dynamic activation of stress kinases, such as ERK1/2 and Akt, is believed to be protective, attenuating contractile dysfunction and infarct size (22). In this study, phosphorylation of ERK1/2 in response to ischemia-reperfusion was similar in magnitude in both diet groups, whereas phosphorylation of Akt in response to ischemia-reperfusion was nonsignificant in both groups. Thus, the data do not identify differences in dynamic activation of these stress kinases as accounting for differences in functional recovery between the groups. However, tissue sampling at 120 min of reperfusion may have been too late to detect differences in dynamic activation between groups, as peak activation of stress kinases has been shown to occur ~30 min after reperfusion in another porcine study (48).

Although dynamic activation of Akt by ischemia was not identified in this model, tonic activation of Akt in metabolic syndrome hearts was suggested by higher levels of the phosphorylated form in both nonischemic and ischemic-reperfused regions compared with the corresponding regions of control hearts. Similarly, we have observed increases in Akt phosphorylation in skeletal muscle and the thoracic aorta of metabolic syndrome pigs (27, 58). Tonic activation of Akt may be deleterious to cardiac function. Mice with cardiac-specific constitutively active Akt exhibit greater susceptibility to ischemia-reperfusion injury, associated with ventricular hypertrophy, feedback inhibition of PI3K, and impaired insulin signaling (33, 38), all features also observed in the present porcine model of metabolic syndrome (27). There is precedent for the tonic activation of Akt by high-fat diet feeding: insulin-resistant rats fed a high-fat diet for 8 wk or obese mice fed a high-fat diet for 6 mo exhibit elevated basal Akt activity and blunted insulin-stimulated Akt activation, possibly related to increased plasma insulin concentration (17, 39), as found in the present model of metabolic syndrome. Potentially deleterious downstream effects of chronic Akt activation include the redistribution of fatty acid translocase CD36 to plasma membrane, where it may promote fatty acid uptake, and inactivation of the forkhead transcription factor Foxo3a, potentially promoting hypertrophy (17, 39).

**Limitations.** Findings in this study were made under anesthetized, open-chest conditions that are known to exacerbate myocardial stunning compared with conscious, chronically instrumented conditions (55); nonetheless, the present findings may bear direct clinical relevance to myocardial ischemia occurring in the setting of cardiac surgery. Attenuation of cardioprotective signaling has been observed in hearts of aged rodents compared with younger rodents (6). It is uncertain whether the differences observed between juvenile pigs with or without metabolic syndrome would also be observed in mature or aged pigs.

**Summary and clinical implications.** Metabolic syndrome affects a broad and growing swath of the population and has a potent, negative clinical interaction with ischemic heart disease (36). The large animal model used in the present study recapitulates many of the characteristics of clinical metabolic syndrome but avoids the development of atherosclerosis, allowing metabolic effects to be evaluated independently. Our results demonstrate that the porcine metabolic syndrome heart is particularly vulnerable to contractile and metabolic dysfunction after ischemia and reperfusion and that multiple mechanisms likely contribute to this vulnerability. The findings in this study may help to explain the poor prognosis of patients with metabolic syndrome who suffer an acute coronary event.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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