Animal care and use. All protocols involving animals were approved by an Institutional Animal Care and Use Committee and complied fully with recommendations in the *Guide for the Care and Use of Laboratory Animals* [21] and the American Veterinary Medical Association Panel on Euthanasia [22]. Male Ossabaw swine at the age of seven months (sexually mature adult) were assigned to three diet groups for 55 weeks before stenting. Lean control swine (Lean, N=9) were fed lean chow containing 22% kcal from protein, 70% kcal from carbohydrates, and 8% kcal from fat. Pigs in the lean group ate 2500 kcal/day. Sedentary MetS (N=9) and exercise trained (XMetS, N=9) groups were fed a high fat/2% cholesterol atherogenic diet composed of lean chow supplemented with (percent by weight): cholesterol 2.0, hydrogenated soybean oil 16.7 (contains 56% trans fatty acids), corn oil 2.5, and sodium cholate 0.7. This mixture yielded a composition of 11% kcal from protein, 43% kcal from carbohydrates and 46% kcal from fat. Atherogenic diet groups ate 6000 kcal/day until sacrifice. All animals were housed in individual pens and provided a 12-hr light/12-hr dark cycle. Water was provided ad libitum.

Exercise Training. Pigs randomized to the exercise group began treadmill training 4 weeks prior to initial cardiac catheterization. To prevent any detrimental cardiac event in obese, MetS swine with CAD and after stenting, the endurance exercise training protocol was modified from our previous studies (e.g. [15]). This protocol complied fully with guidelines in the "*APS Resource Book for the Design of Animal Exercise Protocols"* [23]. Briefly, exercise training consisted of treadmill running performed 4 days/week. During the first week of training, pigs were acclimated to the treadmill at 4 km/h (endurance) with 0% grade for 20-30 minutes and at 5.6 km/h for 15 minutes. After acclimation the daily 45 min training bout consisted of four stages: 1) a 5 min warm-up at 2.2 km/h, 2) 5 min at 6.1 km/h (~40-50% maximum heart rate), 3) 30 min at 7.7 km/h with a variable grade (~65-75% of maximum heart rate), and 4) a 5 min cool down at 3.5 km/h. Heart rates were monitored continuously using an external monitor (T51H,

Polar,Lake Success, NY). Throughout the total 7 week training period, training intensity was maintained in the desired heart rate range by altering the treadmill grade. Each pig resumed exercise training 48 hours following coronary stenting procedure and this regimen was maintained for 3 weeks following stenting before repeat cardiac catheterization and euthanasia.

Intravenous glucose tolerance test (IVGTT). Swine were acclimatized to low-stress restraint in a specialized sling for 5-7 days before the IVGTT was conducted [17-19]. Swine were then fasted overnight, and anesthetized with isoflurane (maintained at 4% by mask with supplemental O_2). The right jugular vein was catheterized percutaneously. Following catheterization, swine were allowed to recover for 3 h before the IVGTT to avoid any effect of isoflurane on insulin signaling [19]. For IVGTT, conscious swine were restrained by sling and baseline blood samples were obtained. Glucose (1g/kg body weight; *i.v.*) was administered and timed blood samples were collected [19]. Blood glucose was measured using YSI 2300 STAT Plus Glucose analyzer. Plasma insulin assays were performed by Linco Research Laboratories (St. Charles, MO).

Plasma lipid assays. Venous blood samples were obtained following overnight fasting. Fasting samples were analyzed for triglyceride and total cholesterol [fractionated into high density lipoprotein (HDL) and low density lipoprotein (LDL) components] [17]. Cholesterol in lipoprotein fractions was determined after precipitation of HDL using minor modifications of standard methods [24]. Specifically, apolipoprotein-B-containing lipoproteins were precipitated with heparin-$MnCl_2$ and the supernatant was assayed. LDL was calculated from the Friedewald equation: LDL = total cholesterol – HDL – (triglyceride ÷ 5).

Stent procedure. Procedures were similar to previous reports [18,25,26]. Swine received 325 mg aspirin and 25 mg Plavix (clopidogrel) daily as anti-platelet therapy which began one day prior to the stent procedure and continued for the duration of the

study. Following an overnight fast, swine received (in mg/kg; *i.m.*) 0.05 atropine, 2.2 xylazine, and 5.5 telazol. Swine were intubated and anesthesia was maintained with isoflurane (2-4%, with supplemental O_2). The isoflurane level was adjusted to maintain anesthesia with stable hemodynamics. Heart rate, aortic blood pressure, respiratory rate, and electrocardiographic data were continuously monitored throughout the procedure. Under sterile conditions, a 7F vascular introducer sheath was inserted into the right femoral artery and heparin (200 U/kg) administered. A 7F guiding catheter (Amplatz L, sizes 0.75-2.0; Cordis) was advanced to engage the left main coronary ostium. A 3.2F, 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/sec. Video images were analyzed off-line (Sonos Intravascular Imaging System; Hewlett Packard). The IVUS catheter was removed and a coronary stent (2.5-4.0 mm diameter by 8 mm length, Express2; Boston Scientific) catheter was deployed. Stent diameter was chosen to match artery diameter using optimal balloon inflation pressure. Two stents were deployed in each animal. One stent was placed in the circumflex artery (CFX), while the second stent was placed in the left anterior descending artery (LAD). Angiography was performed to ensure proper longitudinal stent placement and IVUS was repeated to confirm deployment of the stent to the proper arterial lumen diameter (1.0x normal artery reference) [18,25,26]. The IVUS catheter, guide catheter, and introducer sheath were removed and the right femoral artery ligated. The incision was closed and the animal was allowed to recover. Cephalexin (1000 mg) was given twice a day for six days following the stent procedure.

Intra-stent histology. Stented coronary segments were placed in zinc-buffered formalin at sacrifice for histological analysis. Stent histology was performed by MicaGenix Corp. (Indianapolis, Indiana, USA, as we have described previously [27]. Images were captured with a Nikon CoolPix 990 (3.34 MegaPixel) digital camera.

Measurements were made with Image Pro Plus v.4.1 software (Media Cybernetics, Silver Springs, Maryland, USA). Collagen content in the media and intima was determined as we previously described [27]. Cell count was assessed using hematoxylin and eosin.

Cell dispersion. The procedure for the isolation of the right coronary artery and the enzymatic dispersal of porcine coronary smooth muscle cells has been previously described [9,15]. Briefly, arteries were freshly dissected and smooth muscle cells dispersed. Arteries were incubated in a low calcium solution containing collagenase for 45 minutes to disperse endothelial cells, followed by a second period of 30 minutes to isolate the smooth muscle cell fraction.

Intracellular Ca^{2+} measurements. Whole cell intracellular Ca^{2+} levels were obtained at room temperature (22–23°C) using the fluorescent Ca^{2+} indicator, fura-2, and the InCa++ Ca^{2+} Imaging System (Intracellular Imaging, Cincinnati, OH) as previously described by our laboratory [9,14,15].

Patch clamp electrophysiology. All electrophysiological experiments were performed using a Axopatch 200B integrating patch-clamp amplifier and a DigiData 1440A analog-digital converter and analyzed using Axon PCLAMP 10 software package. Cells were voltage-clamped at a holding potential of -60 mV and 300 ms voltage ramps from -100 to +100 mV were applied at 5 sec intervals similar to previous protocols [28]. The pipette solution contained (mM): 135 CsMeSO3, 10 CsCl, 2 MgCl2, 10 EGTA, 20 HEPES (pH 7.2). Extracellular solutions contained (mM): 150 NaCl, 2 MgCl2, 1.2 CaCl2, 5.5 glucose, 10 HEPES (standard extracellular solution, pH 7.2) or divalent cation free solution having: 150 NaCl, 1 EGTA, 5.5 glucose, 10 HEPES (pH 7.2) and 5 mM caffeine added where indicated.

Reverse transcription-polymerase chain reaction (RT-PCR). The total RNA from pig coronary arteries was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA).

The full-length transcripts of TRPC1 were amplified using the one-step SuperScript RT-PCR platinum Taq HiFi system (Invitrogen, Carlsbad, CA). Primers for PCR amplification of TRPC1 were designed for the Ossabaw TRPC1 sequence and were as follows: forward, ATGGCGGCCCTGTACCCGAG; reverse: CATAGCATATTTAGAAGTCCGAAAGCC. The transcripts were confirmed to be the pig TRPC1 by sequencing.

Real Time RT-PCR (or Quantitative RT-PCR) analysis: BioRad iScript cDNA Synthesis kit was used to reverse transcribe cDNA using total RNA isolated from coronary of 18 pigs (6 Lean, MetS, and XMetS) as templates. The Applied Biosystems 7500 Real Time PCR System was used and data were quantified using comparative Ct (ddCt) method. The endogenous control (18S rRNA) was amplified using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), whereas TRPC/Orai/Stim was amplified using SYBR Green Master mix (Applied Biosystems, Foster City, CA). The dissociation curves were run for all completed SYBR Green reactions to rule out non-specific amplifications and primer-dimers. The non-template control was performed as negative control.

Immunoblots. Protein extracts were subjected to 10.5% SDS-PAGE separating gel (4% stacking gel) and transferred to nitrocellulose membranes. The membrane (blocked with 5% casein) was incubated with the primary antibodies and then with an anti-rabbit or mouse HRP-conjugated antibody (Pierce, Rockford, IL, 1:20,000). Bound HRP-conjugates were detected using a SuperSignal West Femto Kit (Pierce), according to the manufacturer's instructions. The monoclonal anti-TRPC1 antibody was a gift from Dr.Tsiokas (University of Oklahoma Health Sciences Center). STIM1 and ORAI1 antibodies were from Alomone labs (Rehovet, Israel).

Assessment of coronary artery disease (atherosclerosis). IVUS pullbacks performed during the stenting procedure and before stent placement were used to

assess native atheroma [16,26]. Measurements were obtained every 2 mm through the length of the artery. Each cross-sectional IVUS image was divided into 16 equal segments. Percent circumferential wall coverage was calculated as (# segments containing atheroma ÷ 16) x 100%, similar to previous reports [17,26].