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ORIGINAL CONTRIBUTION

Severe familial hypercholesterolemia impairs the regulation of coronary blood flow and oxygen supply during exercise

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Abstract Accelerated development of coronary atherosclerosis is a defining characteristic of familial hypercholesterolemia (FH). However, the recent data highlight a significant cardiovascular risk prior to the development of critical coronary stenosis. We, therefore, examined the hypothesis that FH produces coronary microvascular dysfunction and impairs coronary vascular control at rest and during exercise in a swine model of FH. Coronary vascular responses to drug infusions and exercise were examined in chronically instrumented control and FH swine. FH swine exhibited ~tenfold elevation of plasma cholesterol and diffuse coronary atherosclerosis (20–60 % plaque burden). Similar to our recent findings in the systemic vasculature in FH swine, coronary smooth muscle nitric oxide sensitivity was increased in vivo and in vitro with maintained endothelium-dependent vasodilation in vivo in FH. At rest and during exercise, FH swine exhibited increased myocardial O_2 extraction resulting in reduced coronary venous SO_2 and PO_2 versus control. During exercise in FH swine, the transmural distribution of coronary blood flow was unchanged; however, a shift toward anaerobic cardiac metabolism was revealed by increased coronary arteriovenous H^+ concentration gradient. This shift was associated with a worsening of cardiac efficiency (relationship between cardiac work and O_2 consumption) in FH during exercise owing, in part, to a generalized reduction in stroke volume which was associated with increased left atrial pressure in FH. Our data highlight a critical role for coronary microvascular dysfunction as a contributor to impaired myocardial O_2 balance, cardiac ischemia, and impaired cardiac function prior to the development of critical coronary stenosis in FH.

Keywords Swine · Atherosclerosis · Cardiac · Microspheres · Microvascular Dysfunction

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Introduction

Familial hypercholesterolemia (FH) is the most common monogenic disorder of lipoprotein metabolism resulting in pronounced lifetime hypercholesterolemia with plasma low-density lipoprotein (LDL) levels ranging between 190 and 1100 mg/dl [31]. Recent reports have nearly doubled prior estimates of FH prevalence to 1 in 200–300 individuals worldwide and have drawn attention to the underdiagnosis and undertreatment of this disorder, still considered by many clinicians to be rare [9, 21, 40]. FH is associated with markedly increased cardiovascular risk, particularly due to accelerated coronary atherosclerosis [31, 40]. A thorough understanding of the mechanistic basis of coronary mortality associated with FH, however, is lacking.

Accumulating evidence recognizes coronary microvascular dysfunction as an important factor contributing to cardiovascular mortality associated with co-morbid conditions, such as hypercholesterolemia and atherosclerosis [13, 41, 43, 52]. Recent clinical studies highlight the relationship of coronary microvascular dysfunction to poor outcomes during the progression of coronary atherosclerosis in patients with "intermediate" (40–70 % luminal narrowing) coronary artery stenosis [52]. Specifically, impaired coronary flow velocity reserve, an index of microvascular function, is associated with a significantly increased major adverse cardiovascular event rate in patients with "intermediate" coronary artery stenosis [52]. Similarly, reduced coronary flow reserve has been reported in patients with FH in the absence of flow-limiting coronary stenosis [30, 38, 54]. Indeed, a central role for coronary microvascular dysfunction owing to co-morbidity-associated systemic inflammation has recently been proposed to underlie the development of cardiac diastolic dysfunction [41]. Animals and humans with hypercholesterolemia demonstrate impaired coronary arteriolar dilation to pharmacologic agents in vivo [10, 38, 55] and ex vivo [25, 29, 46, 48, 53] that precede atherosclerotic plaque development [10, 55]. Taken together, these data challenge the stenosis-centered paradigm of cardiac ischemia in hypercholesterolemia and FH and highlight a need for a better understanding of coronary vascular function and blood flow control prior to the development of a critical coronary stenosis.

We recently reported, in chronically instrumented animals, impaired systemic and pulmonary vascular function in vivo during exercise in the Rapacz swine model of FH owing to endothelial dysfunction [2, 8]. These swine recapitulate human FH and its consequences (i.e., coronary atherosclerosis) due to reduced LDL catabolism and defective receptor binding of buoyant LDL [7, 32, 42, 44]. Given the lack of information regarding in vivo coronary blood flow control in response to physiologically relevant stressors, we utilized this model to evaluate coronary flow control in the presence of non-flow-limiting stenosis during exercise in FH. Specifically, we tested the hypothesis that FH produces coronary microvascular endothelial dysfunction and impairs coronary vascular control at rest and particularly during exercise compared to normal swine.

Methods

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Missouri and thus were performed in accordance with the Declaration of Helsinki (1964) and its later amendments. Adult (1–2 years) control, normolipidemic (Con, $n = 7$; 25–60 kg), or Rapacz familial hypercholesterolemic (FH, $n = 4$; 80–100 kg) swine were utilized. FH swine were obtained from the University of Wisconsin Swine Research and Teaching Center. Control swine were fed standard chow (by weight 16.7 % protein, 2.6 % fat, 53.2 % carbohydrate; 2.57 kcal/g). FH swine were fed standard chow until 14 mo of age when they were switched to a high-fat chow (by weight 13 % protein, 21.3 % fat, and 41.4 % carbohydrate; 5.14 kcal/kg) for 5 mo prior to instrumentation. Daily caloric intake was ~51 kcal/kg for control swine and ~48 kcal/kg for FH swine. FH swine were maintained on high-fat chow following instrumentation and throughout experimental protocols.

Instrumentation

Daily adaptation of animals to laboratory conditions and treadmill running began 1 week prior to surgery. Animals were sedated with telazol (5 mg/kg, im) and xylazine (2.2 mg/kg, im), intubated and ventilated with 1–3 % (vol/vol) isoflurane in the air to induce and maintain anesthesia. Body temperature was maintained between 36.5 and 37.5 °C using a warming blanket, and ECGs were monitored from the standard limb leads. Under sterile conditions, the chest was opened via the third intercostal space, and a fluid-filled polyvinylchloride catheter was inserted into the aortic arch for mean arterial pressure (MAP) measurement, blood sampling for PO_2, PCO_2, pH, O_2 saturation, and hemoglobin concentration, the computation of O_2 content, O_2 supply, and O_2 consumption [15, 34], as well as the collection of reference samples for microsphere studies [14]. A fluid-filled catheter was also inserted into the left atrium for measurement of the left atrial pressure (LAP) and infusion of microspheres and in the pulmonary artery for the blood sampling and infusion of drugs. A small angiocatheter was inserted into the anterior interventricular vein for coronary venous blood sampling. Finally, bidirectional transit-time flow probes (Transonic Systems) were placed around either the ascending aorta or pulmonary artery for the measurement of cardiac output and around the proximal left anterior descending coronary artery for the measurement of coronary blood flow (CBF).

Electrical wires and catheters were tunneled subcutaneously to the back, the chest was closed, and animals were allowed to recover. Animals received analgesia [buprenorphine (0.3 mg im) for 2 days] and antibiotic prophylaxis [amoxicillin (25 mg/kg iv) and gentamycin (5 mg/kg iv) for 5 days]. Catheters were flushed daily with physiological saline containing 1500–5000 IU/ml heparin. Studies were performed starting approximately 1 week after surgery, similar to the previous studies [2, 4].

Infusion protocols

The effect of FH on coronary endothelium-dependent and -independent vasodilation was assessed via infusion of the endothelium-dependent vasodilator ATP, the nitric oxide (NO) donor sodium nitroprusside (SNP), and the ATP-sensitive K^+ (K_{ATP}) channel opener bimakalim, similar to the previous studies [8, 14, 35]. With swine standing quietly on the treadmill, resting hemodynamic measurements were collected with the subsequent infusion of agonists. Graded infusions of ATP (100–300 μg kg^{-1} min^{-1} iv), SNP (2–4 μg kg^{-1} min^{-1} iv), and bimakalim (75–225 ng kg^{-1} min^{-1} iv) were performed with each dose infused for 10 min and stable hemodynamics recorded after 6–8 min of each dose.

Exercise protocol

The effect of FH on CBF control at rest and during exercise was assessed via a treadmill exercise protocol. Briefly, with swine standing quietly on the treadmill, resting hemodynamic measurements, blood samples, and rectal temperature were obtained. Subsequently, control swine were subjected to a four-stage treadmill exercise protocol [2–5 miles/h (mph) at 0 % inclination], and FH swine were subjected to a three-stage exercise protocol [1–3 mph at 0 % inclination]. FH swine, in general, would not exercise beyond 3 mph. Hemodynamic variables were continuously recorded digitally on a Codas workstation (ATCODAS, Dataq Instruments, Akron, OH) with blood samples collected during the final 30 s of each 3-min exercise stage when steady-state hemodynamics had been achieved. Blood samples were analyzed for PO_2, PCO_2, pH, O_2 saturation, and hemoglobin concentration (ABL-720, Radiometer, Copenhagen).

Myocardial blood flow

The transmural distribution of myocardial blood flow was measured by the injection of 6–8 × 10^6 microspheres [15 μm in diameter labeled with lanthanum, iridium, europium, holmium, ytterbium, samarium, scandium, lutetium, or terbium stable isotopes (BioPAL, Worcester, MA)] into the left atrium at rest and during exercise at 3 mph in the majority of animals. Injection was performed at 4 mph in five control swine. An arterial blood reference sample was withdrawn at a constant rate of 5 ml/min starting 10 s before the injection of microspheres and continuing for 90 s. At the conclusion of the study, swine were euthanized and the heart was excised and weighed. The left ventricular anterior wall was separated from the heart and divided into four layers of equal thickness from epicardium to endocardium. Samples from each layer were weighed and dried in individual vials along with reference blood samples. Myocardial and reference blood samples were sent to BioPAL for the analysis of microsphere content via neutron activation technology [45]. Blood flow per gram of myocardium (Q_m) was calculated as: $Q_m = Q_r \times D_m/D_r$, where Q_r is the rate of withdrawal of the reference blood sample (in ml/min), D_m is the disintegrations per minute per gram of the myocardial specimen, and D_r is the disintegrations per minute of the reference blood sample.

Intravascular ultrasound (IVUS)

Coronary atherosclerosis was assessed by IVUS prior to sacrifice in FH swine. IVUS was initiated using the standard coronary catheterization techniques as described previously [17, 18, 28, 50, 51]. Briefly, a 7F introducer was inserted in the right femoral artery. Heparin (300 U/kg) was administered following femoral access and maintenance doses given every hour (100 U/kg). A guide catheter (6F) was directed up the aorta and engaged into the left ostium under fluoroscopic guidance. The left anterior descending (LAD) artery was selectively engaged. IVUS pullbacks (0.5 mm/s; Galazy II, Boston Scientific, 40 MHz) were obtained for the proximal, mid, and distal 3 cm segments of the LAD after intracoronary nitroglycerin. The 3D reconstruction of IVUS pullbacks was performed using the QIVUS software (Medis). Percent plaque (i.e., percent vessel volume reduction) was calculated as (plaque volume/vessel volume) × 100 in the proximal, mid, and distal segments.

Isolated arterioles

Apical left ventricular coronary arterioles from control and FH swine (<150 μm internal diameter) were isolated, mounted on glass micropipettes, and pressurized to 60 cmH_2O for determination of vasodilator responses, as previously described [3]. Following 1 h equilibration, arterioles were preconstricted with endothelin-1. Endothelium-dependent and –independent vasodilator responses to bradykinin (BK; 3 fM–10 nM) and sodium nitroprusside (SNP; 1 nM–100 μM), respectively, were determined. Maximal passive diameter was determined at the end of the experiment by replacing the vessel bath with Ca^{2+}-free buffer.

Data acquisition and analysis

All signals were recorded at a sampling rate of 225 Hz, digitized online, and stored on a computer for later post acquisition offline analysis with a program written in MatLab (MathWorks, Natick, MA). Mean values for

in vivo measurements were determined across 10 s at rest and at the end of each exercise level. Systemic and coronary hemodynamic measurements as well as blood gas variables were analyzed, as previously described [15]. MVO_2 was calculated as the product of CBF (normalized per gram of myocardium via blood flows determined by microspheres; CBF g^{-1}) and the difference in O_2 content between arterial and coronary venous blood. Coronary vascular conductance was calculated as the ratio of normalized CBF to MAP. Coronary arteriovenous $[H^+]$ gradient was calculated and presented as coronary venous $[H^+]$ minus arterial $[H^+]$. Cardiac work was calculated as the product of stroke volume, heart rate, and systolic aortic blood pressure divided by the left ventricular weight. Body oxygen consumption was normalized to body weight. Percent maximal dilation of isolated arterioles was calculated as: $[(Dd - Db)/(Dmax - Db) \times 100]$, where Dd is the diameter after a drug concentration, Db is baseline diameter, and Dmax is maximal passive diameter. The statistical analysis was performed using two-way ANOVA followed by Bonferroni post hoc testing, when appropriate, and using linear regression. Phenotype data between strains was analyzed by unpaired *t* tests. A *p* value <0.05 was considered significant. Data are expressed as mean ± SE.

Table 1 Phenotypes of control and FH swine

	Control	FH
Body weight (kg)	41 ± 4	84 ± 4*
Heart weight (g)	280 ± 11	411 ± 6*
HW:BW (g kg^{-1})	5.8 ± 0.3	4.9 ± 0.2*
Total cholesterol (mg dl^{-1})	69 ± 12	644 ± 139*
Triglycerides (mg dl^{-1})	65 ± 19	55 ± 10
Glucose (mg dl^{-1})	86 ± 16	87 ± 5

Values are mean ± SE

* $p < 0.05$ versus Control

Results

Phenotype of control and FH swine

Phenotypic characteristics of control and FH swine are presented in Table 1. FH swine had higher body and heart weights, but a lower heart weight-to-body weight ratio compared to control swine. As expected, FH swine exhibited significant elevations in total cholesterol, but not triglycerides or glucose, compared to control swine. We have previously reported that the elevation in total cholesterol in this model is due primarily to dramatic increases in low-density lipoprotein cholesterol with a modest elevation of high-density lipoprotein cholesterol [2, 8]. FH swine exhibited diffuse, but not critical (i.e., >70 % luminal narrowing), atherosclerosis burden along the LAD, assessed by IVUS (Fig. 1).

Coronary vasodilator function in control and FH swine

Coronary vasodilator function was assessed via changes in coronary vascular conductance (CVC) in response to the infusion of endothelium-dependent (ATP) and -independent (SNP and bimakalim) vasodilators. Systemic hemodynamic responses to drug infusions were not different between groups (Table 2). Surprisingly, ATP-induced coronary vasodilation was not different between control and FH swine (Fig. 2a). However, coronary vasodilation to SNP, an index of smooth muscle NO sensitivity, was increased in FH swine compared to control (Fig. 2b), suggesting that a reduced bioavailability of NO was compensated for by increased smooth muscle sensitivity to NO. Vasodilation in response to the K_{ATP} channel opener bimakalim was not different between these two strains (Fig. 2c).

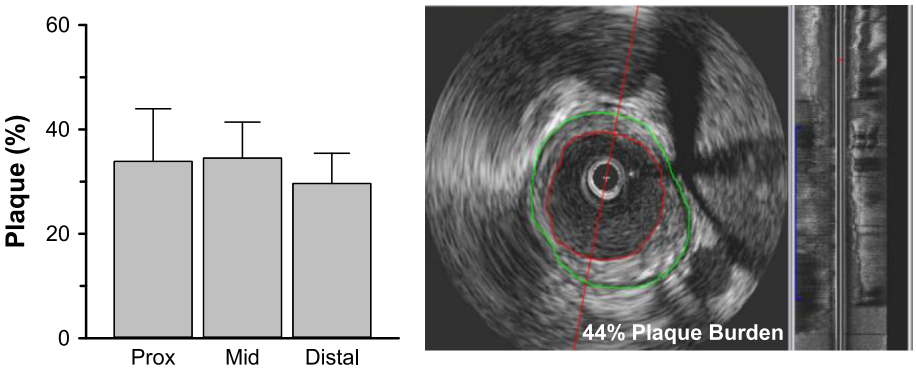

Fig. 1 Coronary atherosclerosis in the left anterior descending (LAD) coronary artery of FH swine, assessed by IVUS. Atherosclerosis burden (percent vessel volume reduction) was assessed in proximal (Prox), mid, and distal LAD in FH swine. Representative IVUS pullback of the LAD coronary artery (*right panel*) with discrimination of the vessel wall (*green*) and luminal border of the atherosclerotic plaque (*red*). Values are mean ± SE

Table 2 Systemic hemodynamic responses to drug infusions in control and FH swine

		Standing	ATP (μg kg^{-1} min^{-1})		
			100	200	300
HR (beats min^{-1})	Control	95 ± 4	106 ± 9	124 ± 9	132 ± 8*
	FH	110 ± 8	134 ± 9†	145 ± 8*	139 ± 4
MAP (mmHg)	Control	112 ± 4	107 ± 5	97 ± 5	86 ± 6*
	FH	104 ± 7	106 ± 7	92 ± 11	85 ± 5
		Standing	SNP (μg kg^{-1} min^{-1})		
			2	3	4
HR (beats min^{-1})	Control	124 ± 8	124 ± 5	141 ± 7	148 ± 4
	FH	115 ± 11	124 ± 7	147 ± 6	147 ± 15
MAP (mmHg)	Control	94 ± 7	90 ± 7	85 ± 5	77 ± 5
	FH	102 ± 9	92 ± 9	87 ± 5	86 ± 6
		Standing	Bimakalim (ng kg^{-1} min^{-1})		
			75	150	225
HR (beats min^{-1})	Control	102 ± 8	112 ± 8	131 ± 8	165 ± 11*
	FH	107 ± 2	116 ± 16	147 ± 19	161 ± 11*
MAP (mmHg)	Control	107 ± 6	108 ± 6	104 ± 5	98 ± 2
	FH	108 ± 5	104 ± 6	97 ± 4	90 ± 6

Values are mean ± SE, $n = 4$–7

HR heart rate, *MAP* mean arterial pressure, *SNP* sodium nitroprusside

* $p < 0.05$ versus standing within group; $^{\dagger}p < 0.05$ versus identical condition in control group

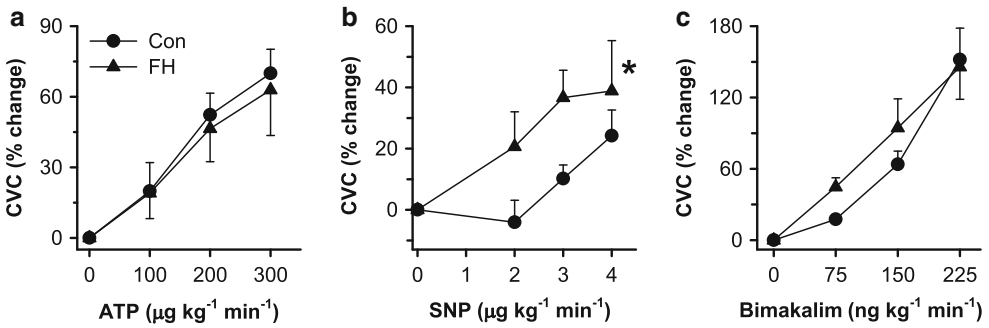

Fig. 2 In vivo coronary vasodilator responses in control and FH swine. Percent increases in coronary vascular conductance (CVC) (i.e., coronary vasodilation) were assessed during infusion of **a** endothelium-dependent vasodilator ATP, **b** endothelium-independent nitric oxide donor sodium nitroprusside (SNP), and **c** endothelium-independent ATP-sensitive potassium channel (K_{ATP}) opener bimakalim. Values are mean ± SE, $n = 4$–7, *$p < 0.05$ main effect FH versus control

Endothelial dysfunction with increased smooth muscle NO sensitivity in FH was confirmed in isolated arterioles demonstrating reduced vasodilation to the endothelium-dependent dilator BK (Fig. 3a) and increased dilation to SNP (Fig. 3b) in FH. Maximum passive internal diameters were similar for arterioles from control and FH swine (102 ± 4 versus 110 ± 7 μm).

Myocardial O_2 balance in control and FH swine

The impact of FH on myocardial O_2 balance was assessed during graded treadmill exercise in control and FH swine. Treadmill exercise increased heart rate, systolic arterial pressure, and body oxygen consumption per kg body weight at comparable treadmill speeds (2 and 3 mph) in

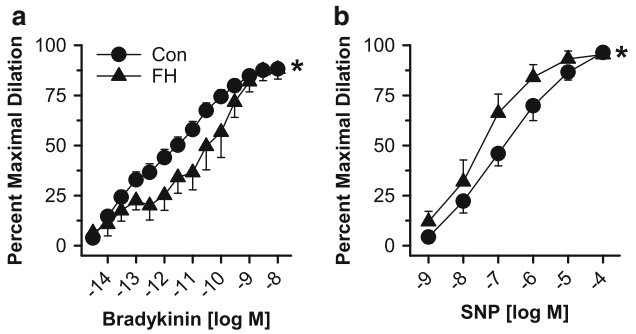

Fig. 3 In vitro coronary arteriolar vasodilator responses in control and FH swine. Vasodilation of isolated, pressurized coronary arterioles was determined to bradykinin and SNP. Values are mean ± SE, $n = 41$ (Con bradykinin), 10 (FH bradykinin), 16 (Con SNP), and 5 (FH SNP), $^{*}p < 0.05$ main effect FH versus control

control and FH swine (Table 3). FH swine, in general, would not exercise beyond 3 mph and exhibited increased left atrial pressures at rest and during exercise (Table 3), as previously reported [2, 8].

Exercise-induced increases in myocardial O_2 demand (i.e., MVO_2 per gram of myocardium) in control swine were slightly exceeded by the increase in CBF g^{-1} (Tables 3, 4), allowing a small decrease in myocardial O_2 extraction (MO_2ex), resulting in small increases in SO_2cv and PO_2cv (Fig. 4). This observation is consistent with the lack of significant α-adrenergic control of the porcine coronary microcirculation [11, 47]. Myocardial arteriovenous H^+ concentration gradient remained relatively constant during exercise. Coronary venous (cv) O_2 content increased in control swine during exercise likely due to the combined effect of increased CBF g^{-1}, increased hematocrit (i.e., arterial Hb; Tables 3, 4), and the small reduction in MO_2ex (Fig. 4).

Resting MVO_2 g^{-1} was similar between FH and control swine (Table 3) and, in general, FH swine exhibited increased MO_2ex, reduced SO_2cv, and reduced coronary venous O_2 content compared to control swine (Fig. 4). During exercise, FH swine had higher MVO_2 g^{-1} compared to control swine at 3 mph (Table 3) that was matched by a similarly elevated CBF g^{-1} (Table 3) such that MO_2ex, SO_2cv, PO_2cv, and coronary venous O_2 content remained constant during exercise (Fig. 4). Importantly, and in contrast to control swine, FH swine exhibited an increase in the myocardial arteriovenous H^+ concentration gradient at 3 mph consistent with a shift toward anaerobic metabolism due to cardiac ischemia (Table 4; Fig. 4). Further analysis revealed that the myocardial arteriovenous H^+ concentration gradient at 3 mph in FH swine correlated with the plaque burden of the mid-LAD coronary artery (Fig. 4).

Table 3 Systemic and coronary hemodynamic responses to exercise in control and FH swine

		Standing	Exercise level (mph)				
			1	2	3	4	5
Systemic hemodynamic							
HR (beats min^{-1})	Con	97 ± 4		172 ± 8*	209 ± 8*	240 ± 6*	252 ± 6*
	FH	140 ± 8†	152 ± 10	173 ± 6*	217 ± 2*		
SAP (mmHg)	Con	114 ± 7		141 ± 10*	160 ± 8*	171 ± 8*	181 ± 8*
	FH	114 ± 5	113 ± 5	127 ± 6	156 ± 9*		
MAP (mmHg)	Con	94 ± 5		109 ± 7	115 ± 6	122 ± 6*	122 ± 5*
	FH	90 ± 7	89 ± 6	98 ± 8	112 ± 11		
LAP (mmHg)	Con	0.9 ± 1.8		3.8 ± 1.4	6.6 ± 1.4*	8.2 ± 1.9*	9.0 ± 2.5*
	FH	9.2 ± 0.9†	10.0 ± 0.5	11.8 ± 1.2†	13.3 ± 1.9†		
BVO_2 (mmol min^{-1} kg^{-1})	Con	0.25 ± 0.02		0.70 ± 0.07*	1.00 ± 0.08*	1.25 ± 0.14*	1.50 ± 0.09*
	FH	0.40 ± 0.03	0.51 ± 0.02*	0.68 ± 0.06*	0.97 ± 0.05*		
Coronary hemodynamics							
CBF (ml min^{-1})	Con	57 ± 7		88 ± 12	109 ± 11*	146 ± 13*	154 ± 14*
	FH	104 ± 10†	117 ± 16	141 ± 14*†	198 ± 27*†		
CBF g^{-1} (ml min^{-1} g^{-1})	Con	1.2 ± 0.2		1.9 ± 0.4	2.3 ± 0.4*	3.1 ± 0.4*	3.3 ± 0.4*
	FH	2.0 ± 0.3	2.2 ± 0.4	2.6 ± 0.4	3.8 ± 0.8*†		

Values are mean ± SE, $n = 4$–7

BVO$_2$ body oxygen consumption normalized to body weight, *CBF* coronary blood flow, *CBF* g^{-1} coronary blood flow normalized to CBF per gram of myocardium, *HR* heart rate, *LAP* left atrial pressure, *MAP* mean arterial pressure, *SAP* systolic arterial pressure

* $p < 0.05$ versus standing within group; † $p < 0.05$ versus identical condition in control group

Table 4 Systemic and coronary blood gas responses to exercise in control and FH swine

Standing			Exercise level (mph)				
			1	2	3	4	5
Hb (g dl^{-1})	Con	8.4 ± 0.8		10.1 ± 0.7	10.8 ± 0.4*	10.6 ± 1.0	11.4 ± 0.4*
	FH	8.7 ± 0.7	9.4 ± 0.6	10.1 ± 0.4	10.8 ± 0.7		
Arterial PO_2 (mmHg)	Con	96.6 ± 3.0		97.5 ± 1.9	97.8 ± 1.8	97.0 ± 5.4	92.3 ± 2.2
	FH	92.8 ± 3.4	96.4 ± 5.9	94.4 ± 4.5	94.0 ± 5.5		
Arterial SO_2 (%)	Con	100.0 ± 0.5		99.7 ± 0.5	99.8 ± 0.5	99.4 ± 0.7	98.6 ± 0.6
	FH	99.7 ± 0.4	100 ± 0.6	100.0 ± 0.8	99.8 ± 0.8		
SO_2cv (%)	Con	16.5 ± 1.1		17.1 ± 1.1	17.9 ± 0.8	20.2 ± 2.2	19.3 ± 1.8
	FH‡	14.0 ± 1.7	13.5 ± 2.1	14.1 ± 2.2	14.9 ± 2.4		
MVO_2 g^{-1} (μmol min^{-1} g^{-1})	Con	4.9 ± 0.6		9.7 ± 2.1	12.7 ± 1.9*	16.1 ± 2.4*	18.5 ± 1.8*
	FH	9.4 ± 2.3	11.8 ± 3.1	14.6 ± 2.8	22.4 ± 6.1*†		
Arterial pH	Con	7.47 ± 0.01		7.44 ± 0.04	7.46 ± 0.03	7.45 ± 0.02	7.42 ± 0.01
	FH	7.45 ± 0.02	7.47 ± 0.02	7.48 ± 0.02	7.47 ± 0.03		
CV pH	Con	7.41 ± 0.01		7.38 ± 0.03	7.39 ± 0.03	7.38 ± 0.02	7.36 ± 0.02
	FH	7 40 ± 0.02	7.40 ± 0.02	7.40 ± 0.02	7.37 ± 0.03		

Values are mean ± SE, $n = 4$–7

CV coronary venous, *Hb* hemoglobin, MVO_2 g^{-1} myocardial oxygen consumption per gram of myocardium, PO_2 partial pressure of oxygen, SO_2 oxygen saturation

* $p < 0.05$ versus standing within group; † $p < 0.05$ versus identical condition in control group; ‡ $p < 0.05$ for main effect of group only

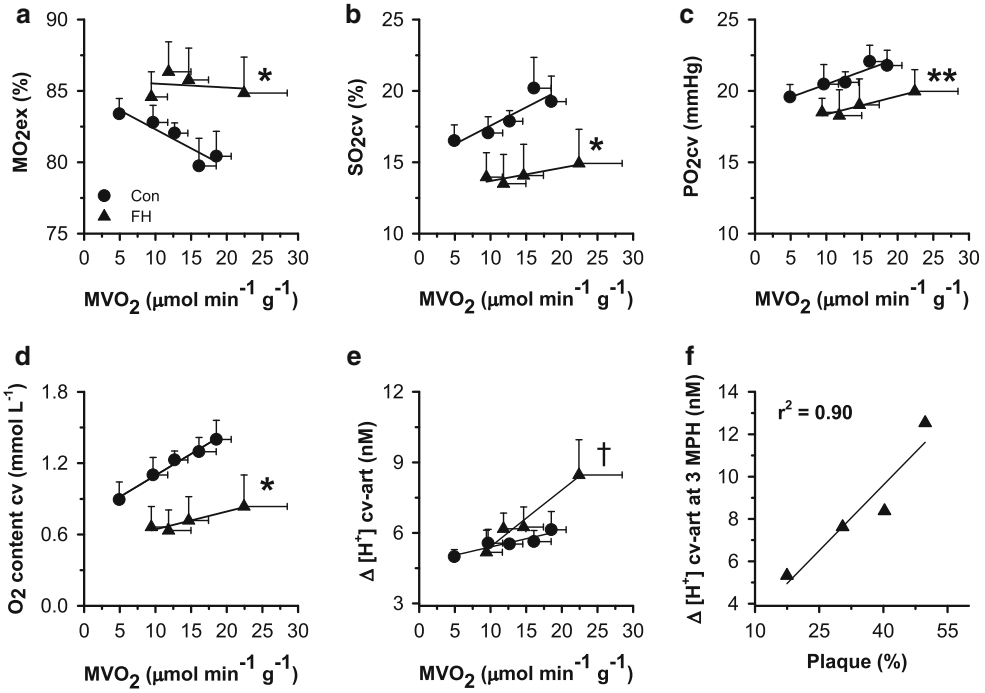

Fig. 4 In vivo coronary resistance vessel tone in control and FH swine. Shown are relations between myocardial oxygen consumption per gram of myocardium (MVO_2) and **a** myocardial oxygen extraction (MO_2ex), **b** coronary venous oxygen saturation (SO_2cv) and **c** tension (PO_2cv), **d** coronary venous oxygen content, and **e** myocardial arteriovenous H^+ concentration gradient (Δ [H^+] cv-art). The arteriovenous H^+ concentration gradient correlates with mid-LAD plaque burden (percent vessel volume reduction) in FH swine, assessed by IVUS (**f**). Values are mean ± SE, *$p < 0.05$ parallel shift versus control, **$p < 0.1$ parallel shift versus control, †$p < 0.05$ rotation versus control

Regional myocardial blood flows in control and FH swine

To assess the impact of FH on the transmural distribution of myocardial blood flows at rest and during exercise, we employed the microsphere technique. The transmural distribution of blood flow at rest and during exercise and the corresponding subendocardial-to-subepicardial blood flow ratios in control and FH swine are shown in Fig. 5a. Resting myocardial blood flows were elevated in FH compared to control swine, but FH swine exhibited a normal subendocardial-to-subepicardial blood flow ratio at

rest. During exercise, myocardial blood flow rates across the LV free wall increased to reach similar levels in control and FH swine resulting in similar subendocardial-to-subepicardial blood flow ratios in both strains during exercise (Fig. 5b).

Cardiac function in control and FH swine

FH swine exhibited relatively higher MVO_2 than control swine, especially at 3 mph (Table 4), so we further examined the relation between cardiac work and MVO_2 (i.e., cardiac efficiency). FH swine exhibited reduced cardiac efficiency compared to control swine that was exacerbated during exercise (Fig. 6a). Our data indicate that FH hearts consume more O_2 at a given level of work than control hearts, which is only partly met by an increase in coronary perfusion and O_2 delivery (Fig. 4). These perturbations were accompanied by a lower stroke volume (indexed to body weight; Fig. 6b) and elevated left atrial pressure in FH swine (Table 3; Fig. 6c).

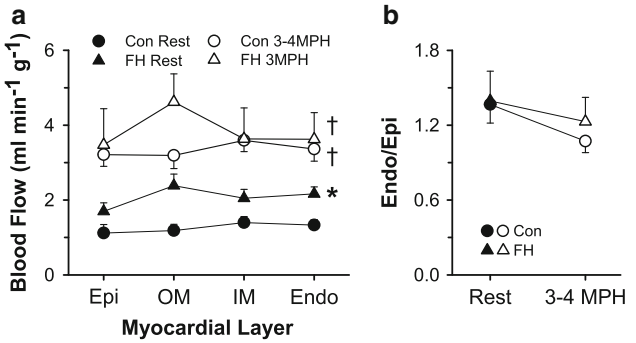

Fig. 5 Myocardial blood flow per gram of myocardium in four layers across the left ventricular free wall at rest and during exercise in control and FH swine. **a** Blood flow to the subepicardium (Epi), outer mid (OM) myocardium, inner mid (IM) myocardium, and subendocardium (Endo) were determined by the infusion of microspheres at rest and during treadmill exercise at 3–4 mph. **b** Subendocardial-to-subepicardial blood flow ratio (Endo/Epi) of the left ventricular free wall in control and FH swine at rest and during exercise at 3–4 mph. Values are mean ± SE, $^{*}p < 0.05$ versus Con Rest, $^{\dagger}p < 0.05$ versus Rest within group

Discussion

This study examined whether FH impairs coronary blood flow regulation and myocardial oxygen delivery. The principle findings of this study are that (1) FH reduced coronary endothelial function due to a reduction in NO bioavailability that was compensated by an increase in coronary vascular smooth muscle NO sensitivity; (2) FH demonstrated perturbations in myocardial oxygen balance that were accompanied by a shift toward cardiac anaerobic metabolism, particularly during exercise, indicated by an increased coronary arteriovenous H^+ concentration gradient; (3) these abnormalities in oxygen supply were not associated with an alteration in the transmural distribution of myocardial blood flow across the left ventricular wall; however, (4) they were associated with a generalized impairment in cardiac contractile efficiency that worsened during exercise in FH.

An association between impaired coronary microvascular function and major adverse cardiovascular events was recently reported in patients with "intermediate" coronary artery stenosis (40–70 % luminal narrowing) [52]. This study and others [6, 13, 37, 41, 43] have challenged the stenosis-centered paradigm of cardiac ischemia in hypercholesterolemia and FH and highlight a growing appreciation for the role of coronary microvascular dysfunction. Indeed, evidence of impaired coronary microvascular function in hypercholesterolemia prior to the development of critical coronary stenosis has been reported [10, 55]. Our results are consistent with these previous reports by demonstrating impaired coronary endothelial function in vivo and in vitro in FH swine with "intermediate" coronary artery stenosis. Specifically, FH resulted in

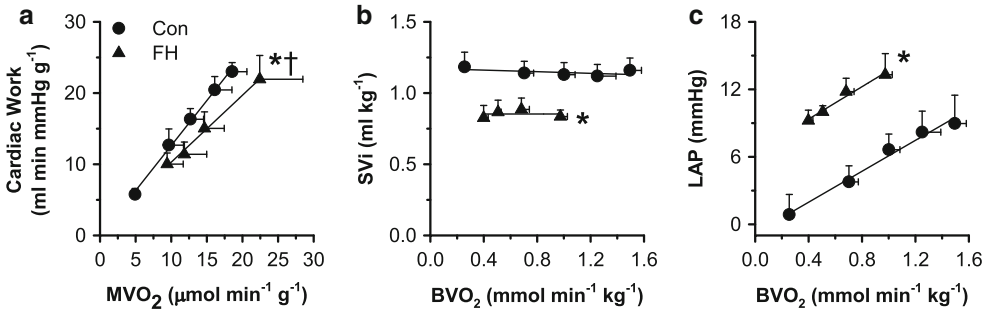

Fig. 6 Cardiac function in control and FH swine. Shown are relations between myocardial oxygen consumption (indexed to myocardial weight) and **a** cardiac work (indexed to left ventricular weight) as well as relations between body oxygen consumption (indexed to body weight) and **b** stroke volume (indexed to body weight) and **c** left atrial pressure. Values are mean ± SE, n = 4–7, $^{*}p < 0.05$ parallel shift versus control, $^{\dagger}p < 0.05$ rotation versus control

increased coronary smooth muscle sensitivity to NO (i.e., increased dilation to SNP) in vivo and in vitro. This was coupled with maintained endothelium- and NO-dependent coronary dilation to ATP in vivo and a rightward shifted dilation to bradykinin with maintained maximal dilation (i.e., reduced sensitivity) in vitro in isolated arterioles. These results may suggest an impairment of endothelium-derived hyperpolarizing factor (EDHF)-induced coronary vasodilation in FH, since bradykinin-induced coronary vasodilation in vitro involves EDHF and NO [26], whereas at the doses examined, ATP-induced coronary dilation in vivo is NO-dependent [14]. Both our in vivo and in vitro results imply that NO-dependent vasodilation is maintained in FH swine by a compensatory increase in NO sensitivity, similar to our previous report in the systemic vasculature of FH swine [8].

Our findings extend prior work by demonstrating that FH alters myocardial O_2 balance at rest and during exercise. Thus, FH swine demonstrated high O_2 consumption for a similar level of cardiac work at rest compared to control swine, which was exacerbated during exercise in FH thereby causing a rotation in the relation between cardiac work and MVO_2. This reduced cardiac efficiency in FH may have resulted from impaired cardiac mitochondrial function. Indeed, cardiac mitochondrial uncoupling has been reported in a similar swine model of moderate coronary atherosclerosis [36] and increased cardiac mitochondrial oxidative stress and enhanced mitochondrial permeability transition pore response has been reported in FH swine [33]. A recent report proposed a critical link between cardiac mitochondrial dysfunction and perturbations in coronary microvascular function in metabolic disease [23]. Consistent with those observations, the present study shows that a higher O_2 consumption in FH swine was not fully met by a commensurate increase in O_2 delivery, necessitating an increase in myocardial O_2 extraction thereby leading to reduced coronary venous PO_2 and SO_2 compared to control swine. The extent of the reduction in coronary venous SO_2 is comparable to that reported previously during exercise in swine with chronic myocardial infarction [12] or metabolic syndrome [5], and reflects perturbations in the regulation of coronary resistance vessel tone [11]. These perturbations were accompanied by a shift toward anaerobic cardiac metabolism (i.e., widening of the coronary arteriovenous H^+ concentration gradient) in FH, an indicator of suboptimal matching of perfusion to the increased MVO_2. These data support a role for impaired myocardial O_2 balance as a contributor to impaired cardiac function in FH, especially during exercise.

Our data revealed a strong relationship between mid-LAD plaque burden and widening coronary arteriovenous H^+ concentration gradient in FH swine during exercise. Given the diffuse nature of atherosclerosis in this model and the potential exacerbation of stenosis severity during exercise [11], we examined transmural myocardial blood flow distribution via microsphere infusion which revealed no shift in the subendocardial-to-subepicardial distribution of coronary blood flow during exercise. Exacerbated stenosis severity during exercise would be expected to cause subendocardial hypoperfusion due to the exercise-induced increase in average subendocardial tissue pressure [1, 11]. Thus, we do not view the relationship between plaque burden and the coronary arteriovenous H^+ concentration gradient as cause-and-effect but rather indicative of similarity in the extent of macrovascular and microvascular disease in this model.

The specific microvascular mechanism(s) in this model are unclear but several observations warrant further investigation. First, microvascular/capillary rarefaction has been reported in skeletal and cardiac muscle in several rodent models of hypercholesterolemia and metabolic syndrome [20, 22, 39, 49]. In skeletal muscle of the obese Zucker rat, microvascular density was inversely proportionate to plasma cholesterol concentration, proportionate to vascular NO bioavailability, and rarefaction was partially prevented by antioxidant treatment to increase NO bioavailability [19, 22]. Thus, FH-associated reductions in NO bioavailability may lead to coronary microvascular rarefaction as a mechanism underlying impaired O_2 supply in this condition. Second, hypercholesterolemia has been shown to blunt adenosine-induced vasodilation [25], a known mechanism of coronary arteriolar dilation in hypoperfused myocardium [11] and a clinically relevant agent for the determination of coronary flow reserve [27, 38]. Thus, while our myocardial blood flow data do not demonstrate selective subendocardial hypoperfusion during exercise, FH swine may exhibit diffuse impairment of adenosine responsiveness contributing to diffuse myocardial ischemia at high MVO_2. Future studies are warranted to explore the potential contribution of these mechanisms to impaired myocardial O_2 balance and exercise-induced ischemia in FH.

A central role for co-morbidity-induced microvascular dysfunction underlying the development of cardiac diastolic dysfunction has recently been proposed. Indeed, although independent of risk factors, a link between impaired coronary O_2 delivery and impaired cardiac ventricular function has been reported in chronically instrumented dogs [16] and swine [24]. In the present study, FH swine exhibited a significant impairment of cardiac efficiency and stroke volume as well as increased left atrial pressures. While our data suggest a role for coronary microvascular dysfunction in impaired cardiac function in FH, the progression of this dysfunction remains uncertain. Specifically, whether stroke volume is low due to diastolic

dysfunction (i.e., due to restricted filling), systolic dysfunction (i.e., due to impaired contractility with maintained high wall stress/MVO_2), or a combination of these requires further examination of cardiac function in this model.

Taken together, our data reveal FH-associated coronary microvascular dysfunction as a primary contributor to impaired myocardial O_2 balance and cardiac efficiency prior to the development of critical coronary stenosis. These data provide insight into potential causes of the substantial risk of major adverse cardiovascular events in patients with "intermediate" coronary artery stenosis by highlighting a role for myocardial ischemia and worsening of cardiac function, particularly during increases in cardiac metabolism (i.e., exercise). Thus, we hypothesize that therapeutic targeting of hypercholesterolemia and/or other associated causes of coronary microvascular dysfunction (i.e., inflammation and oxidative stress) could improve cardiovascular outcomes in patients with FH.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.
The manuscript does not contain clinical studies or patient data.

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