Hypercholesterolemia inhibits L-type calcium current in coronary macro- and not microcirculation

D. K. Bowles,1,2 C. L. Heaps,1 J. R. Turk,1 K. K. Maddali,1 and E. M. Price1,2

1Department of Biomedical Sciences and 2Dalton Cardiovascular Research Center, University of Missouri, Columbia, Missouri 65211

Submitted 17 November 2003; accepted in final form 27 January 2004

Bowles, D. K., C. L. Heaps, J. R. Turk, K. K. Maddali, and E. M. Price. Hypercholesterolemia inhibits L-type calcium current in coronary macro- and not microcirculation. J Appl Physiol 96: 2240–2248, 2004. First published January 29, 2004; 10.1152/japplphysiol.01229.2003.—Hypercholesterolemia (HC) is a primary risk factor for the development of coronary heart disease. Coronary ion regulation, especially calcium, is thought to be important in coronary heart disease development; however, the influence of high dietary fat and cholesterol on coronary arterial smooth muscle (CASM) ion channels is unknown. The purpose of this study was to determine the effect of diet-induced HC on CASM voltage-gated calcium current (ICa). Male miniature swine were fed a high-fat, high-cholesterol diet (40% kcal fat, 2% wt cholesterol) for 20–24 wk, resulting in elevated serum total and low-density lipoprotein cholesterol. Histochemistry indicated early atherosclerosis in large coronary arteries. CASM were isolated from the right coronary artery (>1.0 mm ID), small arteries (~200 μm), and large arterioles (~100 μm). ICa was determined by whole cell voltage clamp. L-type ICa was reduced ~30% by HC compared with controls in the right coronary artery (~5.29 ± 0.42 vs. ~7.59 ± 0.41 pA/pF) but not the microcirculation (small artery, ~8.39 ± 0.80 vs. ~10.13 ± 0.60; arterioles, ~10.78 ± 0.93 vs. ~11.31 ± 0.95 pA/pF). Voltage-dependent activation was unaffected by HC in both the macro- and microcirculation. L-type voltage-gated calcium channel (Ca_l,1.2) mRNA and membrane protein levels were unaffected by HC. Inhibition of ICa by HC was reversed in vitro by the cholesterol scavenger methyl-β-cyclodextrin and mimicked in control CASM by incubation with the cholesterol donor cholesterol:methyl-β-cyclodextrin. These data indicate that CASM L-type ICa is decreased in large coronary arteries in early stages of atherosclerosis, whereas ICa in the microcirculation is unaffected. The inhibition of calcium channel activity in CASM of large coronary arteries is likely due to increases in membrane free cholesterol.

olesterol; vascular smooth muscle; cyclo dextrin; voltage-gated calcium channels; porcine

HYPERCHOLESTEROLEMIA IS A primary causal factor in the development of coronary atherosclerosis. Population studies such as the Framingham Heart Study (39) and the Multiple Risk Factor Intervention Trial (31) have demonstrated a direct relationship between serum total and/or low-density lipoprotein (LDL) cholesterol and coronary heart disease. Accordingly, reductions in serum cholesterol, either pharmacologically or through dietary restrictions, reduce the incidence of coronary heart disease and produce beneficial remodeling of existing lesions (29, 36). The target cells and cellular mechanisms relating hypercholesterolemia and coronary atherosclerosis remain unclear. Alterations in ion regulation, particularly calcium homeostasis, in coronary vascular cells may contribute to the onset and progression of coronary heart disease by producing abnormal vasomotion (21) and/or alterations in gene expression (5). Diet-induced hypercholesterolemia has been reported to alter the activity of several smooth muscle membrane channels and pumps, including voltage-gated calcium channels (VGCC) and Na–K+–ATPase (7, 9), an effect that has been attributed by some to changes in membrane free cholesterol content (34). Conclusions as to whether hypercholesterolemia inhibits or activates membrane channel and transporter activity are varied. Manipulation of membrane free cholesterol content, either through diet or through in vitro enrichment and depletion studies, has often shown an inverse relationship between membrane cholesterol content and channel activity (1, 6, 18, 22, 27). However, VGCC current (ICa) increases have been reported both in cholesterol-enriched cultured cells (28) and in rabbit portal vein smooth muscle after diet-induced hypercholesterolemia (9). Coronary arterial smooth muscle (CASM) cells play a substantial role in coronary lesion formation during coronary heart disease, and VGCCs have been implicated in enhanced coronary vasomotion in coronary disease (21), yet no information is available regarding the effects of hypercholesterolemia on CASM calcium channel function. Therefore, the purpose of this study was to determine the effect of diet-induced hypercholesterolemia on VGCC activity and expression. Furthermore, because coronary atherosclerotic lesion formation is primarily localized in the macro-, not microcirculation, we compared the effects of diet-induced hypercholesterolemia on ICa in CASM from both large conduit and resistance (<300 μm) coronary arteries.

MATERIALS AND METHODS

Animals. Adult male miniature swine weighing 25–40 kg were obtained from the breeder (Sinclair Farms, Columbia, MO) and housed in pens at the College of Veterinary Medicine. Animal protocols were approved by the University of Missouri Animal Care and Use Committee in accordance with the “Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training.”

Diet. Animals were randomly divided into two dietary groups: a group fed a normal pig chow diet (control, n = 17) and a group fed a high-fat, high-cholesterol diet (n = 13). The pig chow fed to the control group was Mini-pig Diet Breeder chow (Purina Lab, St. Louis, MO) containing (by weight) 16.7% protein, 2.6% fat, and 53.2% total carbohydrate (22% kcal from protein, 8% from fat, and 70% from carbohydrate). The hypercholesterolemia diet consisted of Mini-pig Diet Breeder chow supplemented (by weight) with 2.0% cholesterol, 17.1% coconut oil, 2.3% corn oil, and 0.7% sodium cholate (13% kcal

Address for reprint requests and other correspondence: D. K. Bowles, E102 Veterinary Medicine, Univ. of Missouri, Columbia, MO 65211 (E-mail: bowlesd@missouri.edu).

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from protein, 46% from fat, and 41% from carbohydrate). Pigs were fed an average of 15–20 g/kg once per day (20–24 wk). Water was given ad libitum.

**Blood sampling.** Blood samples were collected at baseline, after 4 wk and at 24 wk. Samples were collected from the jugular vein into a vacutainer containing EDTA and centrifuged at 2,000 g for 15 min at 4°C. Plasma was stored immediately at −70°C until analyzed as previously described (33).

**Preparation of coronary arteries.** At 20–24 wk, pigs were anesthetized with ketamine (30 mg/kg) and pentobarbital sodium (35 mg/kg). The hearts were removed and placed in ice-cold (4°C) Krebs bicarbonate solution during vessel isolation. Conduct (>1.0 mm ID) segments of right coronary artery (RCA) were trimmed of fat and connective tissue in sterile MEM containing 20 mM HEPES on ice. Small arteries (225–175 μm ID) and arterioles (75–125 μm ID) were dissected free from the subepicardial wall near the apex. All arteries were stored under sterile conditions in MEM at 4°C until dispersion (0–1 h).

**Cell dispersion.** Freshly dispersed CASM cells were obtained as described previously (2–4, 15). Resistance arteries were incubated in 200 μl of enzyme solution consisting of low-calcium (0.1 mM) physiological solution plus 294 U/ml collagenase (CLS II, Worthington), 6.5 U/ml elastase (Worthington), 2 mg/ml bovine serum albumin (fraction V, Sigma Chemical), 1 mg/ml soybean trypsin inhibitor (type I-S, Sigma Chemical) and 0.4 mg/ml DNase I (type IV, Sigma Chemical). Cells were enzymatically dispersed by incubation for 60 min in a water bath at 37°C. Immediately after, the enzyme solution was replaced with enzyme-free low-calcium solution and isolated single cells were obtained by gentle trituration using a flame-polished Pasteur pipette. For conduit coronary arteries, arteries were opened longitudinally and pinned, lumen side up, onto a silicone rubber substratum in a 2 ml of low-calcium enzyme solution. Cells were enzymatically dispersed for 60 min in a water bath at 37°C. The enzyme solution was then replaced with enzyme-free low-calcium solution, and isolated single cells were obtained by repeatedly directing a stream of low-calcium solution over the artery via a fire-polished Pasteur pipette. All solutions used for conduit and resistance vessels were identical. Cell suspensions were stored in low-calcium (0.1 mM) buffer at 4°C until use (0–6 h).

**Whole-cell voltage clamp.** Whole-cell currents were determined by using a standard whole cell voltage-clamp technique (2–4, 15). Cells were initially superfused with physiological saline solution containing (in mM) 0.1 CaCl₂, 138 NaCl, 1 MgCl₂, 5 KCl, 10 HEPES, and 10 glucose, pH 7.4, during gigaseal formation. After whole cell configuration, the superfuse was switched to physiological saline solution with tetraethylammonium chloride substitution for NaCl and 10 mM Ba²⁺ as the charge carrier. The pipette solution contained (in mM), 120 CsCl, 10 tetraethylammonium chloride, 1 MgCl₂, 20 HEPES, 5 Na₂ATP, 0.5 Tris-GTP, and 10 EGTA, pH 7.1. Ionic currents were amplified by an Axopatch 200B patch-clamp amplifier (Axon Instruments, Foster City, CA). Whole cell currents were low-pass filtered with a cutoff frequency of 1,000 Hz, digitized at 2.5 kHz, and stored on computer. Current densities (pA/pF) were obtained for each cell by normalization of whole cell current to cell capacitance to account for differences in cell membrane surface area. Capacity currents were measured for each cell during 10-ms pulses from a holding potential of −80 mV to a test potential of −75 mV. Capacity currents were filtered at a low-pass cutoff frequency of 5 kHz. Leak subtraction was not performed. Data acquisition and analysis were accomplished using pCLAMP 7.0 software (Axon Instruments). All experiments were conducted at room temperature (22–25°C). Cells were continually superfused under gravity flow.

**Immunoblot.** RCA segments (5-mm axial length) were quick-frozen in liquid N₂ and stored at −80°C until processed. Samples were minced thoroughly in 200 μl of ice-cold buffer (50 mM Tris-HCl, pH 7.4, 0.1 mM EDTA, 0.1 mM EGTA, 0.1% β-mercaptoethanol, 1 mM PMSF, 2 mM leupeptin, 1 mM pepstatin A, 10% vol/vol glycerol) and subjected to 4 × 30 s homogenizations at a speed of 4.0–5.0 using a FASTPREP 120 system (QiBiogene) with samples kept on ice for 5 min between each pulse. After a low-speed centrifugation, supernatants were subjected to high-speed centrifugation (100,000 g for 1 h). Crude membrane pellets were dissolved by vortexing in homogenization buffer containing 1.0% Triton X-100 at room temperature. Membrane protein concentrations (3–5 μg/μl) were determined by using the Bio-Rad Bradford protein assay kit. Equal protein amounts (30 μg) of sample were electrophoresed on 5% SDS-polyacrylamide gels and transferred to a polyvinylidene difluoride membrane at 30 V for 14–16 h at 4°C. After transfer, membranes were blocked for 1 h in Tris-buffered saline solution containing 5% dry milk plus 0.1% Tween 20 and probed with anti-Caᵥ1.2 antibody (1:200; Alomone) for 2 h at room temperature. Immunoreactive bands were detected by chemiluminescence (ECL, Amersham) and quantified by use of Image J software (NIH).

**Quantitative RT-PCR.** Real-time PCR was performed to simultaneously amplify (“duplex”) the Caᵥ1.2 subunit of the VGCC and the constitutively expressed gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). RCA segments (10-mm axial length) were quick-frozen in liquid N₂ and stored in RNase-free microcentrifuge tubes at −80°C until processed. Arterial segments were pulverized under liquid N₂, and a crude lysate was prepared in lysis buffer containing (in mM) 500 LiCl, 10 EDTA, 5 dithiothreitol, and 100 Tris-HCl with 1% LiDS (pH 8.0) by vigorous agitation (vortex). Poly(A)⁺ RNA was isolated with paramagnetic oligo(dT) polyacrylamide beads [Dynabeads oligo(dT)₅, Dynal] and eluted for first-strand cDNA synthesis using reverse transcriptase (RT) and oligo(dT)₁₂–₁₈ to prime the reaction (Superscript Preamplification System, Invitrogen). All PCR reactions were performed in a laminar flow benchtop hood. cDNA samples were used to perform real-time PCR in a 25-μl volume containing buffer [50 mM KCl, 20 mM Tris·HCl (pH 8.4), 5 mM MgCl₂, 0.15–0.20 μM of each primer or probe, 0.2 mM dNTP, and 2.5 U AmpliTaq Gold DNA polymerase (Roche) on a Cepheid Smart Cycler (model SC1000-1). The PCR reaction was initiated by activation of AmpliTaq Gold polymerase for 1 min at 95°C followed by 50 cycles of 95°C (15 s), 54°C (30 s), and 72°C (30 s) using conditions optimized for MgCl₂ concentration and annealing temperature.

Primers were designed on the basis of published sequences from human VGCC and domestic porcine (Sus scrofa) GAPDH. The primer sets were as follows: Caᵥ1.2; sense, 5'-GAA GTG CCT CAC TGT CGG TC-3'; antisense, 5'–CTG GTC CCA ACA GAA ATG AA-3'; and probe 5'-6-FAM d(ACC TGG AGT TAA CCG GAA CAG CG T) BHQ-1–3; GAPDH; sense, 5'-ATG ATT CTA CCC ATG GCA AA-3'; antisense, 5'-TCC ATT GAT GAC AAG CTT CC-3'; and probe, 5'-ROX-CGC CAG ACT GAA GCG TGA GAA TG-3'. Nucleotide sequencing demonstrated complete homology between human and Yucatan porcine Caᵥ1.2 PCR-amplified products. ROX- and FAM-conjugated fluorogenic probes (Biosearch Technologies) for GAPDH and Caᵥ1.2, respectively, were designed for real-time PCR on the basis of the derived Yucatan nucleotide sequences. For quantification, the Taq polymerase-amplified PCR product from the Caᵥ1.2 subunit was subcloned into a plasmid vector by use of the pCR2.1 TOPO TA cloning kit (Invitrogen) and transformed into competent E. coli, and a standard curve was generated plotting threshold cycle against known quantities of cDNA (Fig. 6). All standards and samples were evaluated in triplicate.

**Immunohistochemistry.** Samples of RCA were fixed in neutral buffered 10% formalin for a minimum of 24 h. Samples were split and one half was frozen, sectioned on a Leica CM 1850 cryostat, and stained with Oil Red O to assess lipid accumulation. The remaining sample was embedded routinely in paraffin and sectioned serially at 5-μm thickness. Sections were floated onto positively charged slides (Fisher), deparaffinized, and stained in citrate buffer at pH 6.0 (Dako S1699) for 30 min to achieve antigen retrieval and then cooled for 30 min. The slides were stained manually with Tris buffer or water wash steps performed after each step. Sections were incubated with...
RESULTS

Body weight and blood lipids. Body weight was significantly greater in animals after 20–24 wk on the hypercholesterolemic diet compared with controls (44.6 ± 2.2 kg vs. 36.56 ± 1.8 kg, respectively). As previously reported for this model (33), plasma total cholesterol increased about eightfold and LDL cholesterol increased about sixfold in pigs fed the high-fat, high-cholesterol diet compared with controls at 20–24 wk (Fig. 1). Plasma triglycerides were unaffected by diet. On the basis of this lipid profile, the high-fat, high-cholesterol diet-fed animals are referred to as hypercholesterolemic.

Diet and CASM cell size. Diet had no effect on whole cell membrane capacitance in cells from conduit arteries, resistance arteries, or large arterioles, indicating a similar cell surface area in CASM from control and hypercholesterolemic swine (Table 1). As previously reported (3), cell membrane surface area decreased as arterial diameter decreased, demonstrating a correlation between CASM membrane surface area and arterial size. Series resistance during voltage clamp was <10 MΩ, unaffected by diet, and similar in all arterial sizes (data not shown).

Effect of hypercholesterolemia on coronary artery histology. Previous studies have demonstrated that our model of hypercholesterolemia is associated with the production of Starry type I–III lesions in the RCA and elevations in both serum and coronary C-reactive protein, macrophage accumulation, and monocyte chemotactic protein 1 (35). The present study further demonstrated a 15-fold increase in coronary lipid deposition in hypercholesterolemia compared with controls (3.83 ± 0.50 vs. 0.25 ± 0.25, P < 0.05; Fig. 2A, left). In contrast, no detectable lipid accumulation occurred in microvessels from either group (Fig. 2A, right). Free cholesterol deposition, as indicated by filipin staining, was significantly greater in large coronary arteries from hypercholesterolemic animals compared with controls (P < 0.05; Fig. 2B). Cholesterol deposition was noted in both the neointima as well as the media. In addition, both endothelial and smooth muscle cells in RCA from hypercholesterolemic pigs demonstrated multifocal positive staining for Ki-67, a marker of proliferation (Fig. 2C). Hypercholesterolemia produced a fourfold increase in smooth muscle cells stained positive for Ki-67 (P < 0.05). Together, these findings are consistent with the development of early atherosclerosis in the RCA of hypercholesterolemic swine.

VGCC in coronary smooth muscle. Figure 3 shows a representative family of currents obtained in conduit CASM from either control (A) or hypercholesterolemic (B) animals. Successive depolarization steps from −60 to +70 mV produced inward currents showing a peak near +20 mV and slow

![Fig. 1. High-fat, high-cholesterol diet produces hypercholesterolemia. Serum total cholesterol (TC; circles), LDL cholesterol (LDLc; triangles) and triglyceride (TG; squares) values from control (solid symbols) and hypercholesterolemic (HC; open symbols) swine during 20–24 wk of the high-fat, high-cholesterol diet. Values are means ± SE (some error bars within symbols). P < 0.05 vs. *prediet (pre).](http://jap.physiology.org/)
inactivation (selected test potentials shown). We have previously shown that CASM from male swine are dominated by L-type current, with no discernable T-type current present in any arterial size or group (2). In the present study, hypercholesterolemia did not alter the dominant expression of L-type current as confirmed by the near complete block of inward current by nifedipine (Fig. 3C). However, the magnitude of both the peak and sustained inward current was decreased ~30% in cells from hypercholesterolemic animals compared with control. Current-voltage relationships demonstrated an overall reduction in inward VGCC current in conduit CASM from hypercholesterolemic animals (Fig. 4A). VGCC current density was unaffected in CASM from either small arteries (not shown) or arterioles (Fig. 4B). Both the membrane potential producing half-maximal activation (V0.5) and voltage-sensitivity (k) were unaffected by diet in both conduit and resistance CASM (Fig. 4, C and D). The effect of hypercholesterolemia on peak I_Ca was attenuated as arterial size decreased (Fig. 5), with only a trend for inhibition in small arteries (~200 μm) and no effect in arterioles (~100 μm). Thus the effect of hypercholesterolemia on VGCC current density is greatest in conduit CASM, with progressively diminishing effects in the microcirculation (Fig. 5).
Fig. 4. Effect of HC on the voltage-gated calcium channel (VGCC) current-voltage relationship. Current-voltage (I-V) relationships are shown for whole cell VGCC current in coronary myocytes from conduit arteries (A) and large arterioles (B) from control (○) and HC (□) animals. Current is plotted as the peak inward current measured during a 400-ms step depolarization to the membrane potential (V_m) indicated from a holding potential of −80 mV. Current is normalized to cell membrane capacitance (pA/pF). Data are means ± SE of 4–5 cells per artery per animal (n values as in Table 1). Repeated-measured ANOVA indicated a significant main effect of HC in conduit arteries but not arterioles. Voltage-dependent activation of currents in CASM is shown from conduit arteries (C) and arterioles (D) from control (○) and HC (□) animals. Data are means ± SE, where relative conductance (g/g_{max}) = peak I_\text{Ba}/g_{\text{max}} (V - E_{rev}); g_{\text{max}} is the maximal conductance obtained from the linear regression of the positive limb of the I-V relationship through the apparent reversal potential (E_{rev}), and I_{\text{Ba}} was obtained from the I-V relationship. The g/g_{max} was fit to a conventional Boltzmann distribution equation, 1/[1 + exp(V_{0.5} - V)/k]], where V_{0.5} is the membrane potential producing half-maximal activation and k is the slope factor. Voltage-dependent activation of currents (V_{0.5}, k) were unaffected by HC in any artery. Responses to HC in small arteries were similar to arterioles (data not shown).

Hypercholesterolemia and Ca_{1.2} expression. The reduction in I_{Ca} in conduit CASM with hypercholesterolemia must result from a reduction in the open probability, unitary conductance, and/or number of channels. To examine the possibility that hypercholesterolemia reduced I_{Ca} by inhibiting the synthesis of VGCCs, we compared both Ca_{1.2} mRNA and protein levels in RCA from both control and hypercholesterolemic animals. Ca_{1.2} primers amplified a single product of predicted size (Fig. 6A), and subsequent sequence analysis confirmed complete homology between the amplified sequence of Yucatan porcine and human Ca_{1.2}. Quantitative PCR demonstrated that Ca_{1.2} mRNA levels were unaffected by hypercholesterolemia, indicating no effect of hypercholesterolemia on Ca_{1.2} gene transcription (Fig. 6C). Potential effects of hypercholesterolemia on Ca_{1.2} translation were examined by immunoblots comparing equal amounts of membrane protein from RCA of control (n = 8) and hypercholesterolemic (n = 7) swine.

Fig. 5. HC inhibits peak voltage-gated calcium current (I_{Ca}) in macro-, not microvessel, CASM. Summary of peak I_{Ca} obtained during I-V normalized to cell surface area (pA/pF) among arterial sizes. Current density was significantly inhibited only in CASM from conduit (>1.0 mm ID) coronary arteries and was unaffected in small (sm.) arteries (175–225 μm) and arterioles (75–125 μm). Data are means ± SE; *P < 0.05 vs. respective control.

Fig. 6. No effect of HC on Ca_{1.2} mRNA expression in conduit CASM. Quantitative PCR of Ca_{1.2} mRNA in RCA. A: Ca_{1.2} PCR products with (+RT) or without (−RT) addition of reverse transcriptase. Ca_{1.2} products were of predicted size (68 bp). Sequencing demonstrated complete homology between porcine and human Ca_{1.2} in the amplified region. bp, 25 bp ladder. B: quantitative PCR standard curve of known amounts of cloned Ca_{1.2} cDNA plotted against threshold cycle (C_t). Linear regression fit of the standard curve produced a slope (2.08), indicating a replication efficiency of 1.08. C: relative Ca_{1.2} gene expression expressed as 2^ΔΔC_t, where the threshold cycle difference (ΔC_t) was determined as C_t_{control} - C_t_{HC} for each sample and normalized to average ΔC_t for control. Ca_{1.2} expression was similar in control (n = 4) and HC (n = 4) swine.
Hypercholesterolemia and Coronary Calcium Current

Reversal of hypercholesterolemic inhibition of $I_{Ca}$ by $\beta$-cyclodextrin. Increased membrane free cholesterol content has been shown to inhibit the activity of a number of membrane ion channels and transporters (18, 22). To test the hypothesis that increased membrane free cholesterol content has resulted from increased free cholesterol, $I_{Ca}$ in isolated CASM from control and hypercholesterolemic swine were compared before and after incubation with the cholesterol scavenger methyl-$\beta$-cyclodextrin (m$\beta$CD) (Fig. 8). Before m$\beta$CD, $I_{Ca}$ was reduced -50% in hypercholesterolemic cells compared with control. Incubation with m$\beta$CD (5 mM, 60 min) completely reversed the inhibition of $I_{Ca}$ by hypercholesterolemia. Furthermore, incubation of CASM from control animals with cholesterol-enriched m$\beta$CD inhibited $I_{Ca}$ to a similar extent as that found in CASM from hypercholesterolemic animals. In cells from control animals, m$\beta$CD had no significant effect, although there was a trend for an increase in current with m$\beta$CD. These data indicate that reductions in CASM $I_{Ca}$ in hypercholesterolemia result from an inhibition of channel activity due to increased plasma membrane free cholesterol.

DISCUSSION

The present study is the first to demonstrate a direct link between diet-induced hypercholesterolemia and alterations in coronary smooth muscle $I_{Ca}$. In large coronary arteries, smooth muscle $I_{Ca}$ was attenuated in a large mammal model of total and LDL hypercholesterolemia, a model similar to hypercholesterolemia in humans (36) and one that mimics the early stages of coronary atherosclerosis (37). Furthermore, our data implicate increased membrane free cholesterol as a primary mechanism mediating this effect. These findings provide novel insights into coronary smooth muscle phenotypic changes in early atherosclerosis.

In humans, elevated serum total and LDL cholesterol greatly increase the risk for atherosclerosis. Increased dietary fat and cholesterol in our swine model produced a robust increase in both total and LDL cholesterol [see Thomas et al. (33) for a more detailed lipid profile of this model]. Compared with the rabbit model of hypercholesterolemia, this increase in both total and LDL cholesterol is more similar to the lipid profile found in hypercholesterolemic humans (19). Serum total and LDL cholesterol levels achieved in our porcine model (~350 and ~200 mg/dl, respectively) are in the range of humans at high risk for atherosclerosis and well above the recommended levels for reducing coronary risk in humans (<200 and <130 mg/dl, respectively).

The atherogenic nature of the cholesterol profile is evidenced by the finding that coronary arteries from hypercholesterolemic swine demonstrate elevated lipid and free cholesterol deposition and increased CASM proliferation (Fig. 2) in addition to previously reported production of Stary type I–III...
lesions, elevations in both serum and coronary C-reactive protein, macrophage accumulation, and increased monocyte chemoattractant protein 1 (35). This pathological profile is indicative of the early stages of atherosclerosis (37). Thus the hypercholesterolemic swine appears to be a useful model for studying coronary arterial changes associated with early atherosclerosis.

The decreased VGCC activity found in CASM of hypercholesterolemic animals is consistent with studies showing an inverse relationship between membrane free cholesterol content and ion channel-pump activity (1, 6, 18, 22, 27). Jennings et al. (18) reported that increasing membrane free cholesterol 67% by cholesterol-enriched cyclodextrin in vitro almost completely abolished L-type VGCC in gallbladder smooth muscle. Interestingly, this attenuation was specific to VGCC as K+ currents were unaffected. Chen et al. (7) found a similar increase in membrane cholesterol reduced Na+ current de 

In conclusion, the present study provides novel, direct electrophysiological evidence that diet-induced hypercholesterol-
emia reduces L-type calcium channel current in coronary smooth muscle of the macro- but not microcirculation. Because this model mimics human hypercholesterolemia and early atherosclerosis, these findings implicate a role for VGCC specifically, and ion channels in general, in CASM phenotypic changes at the onset of coronary heart disease.

ACKNOWLEDGMENTS
We gratefully acknowledge the significant contribution of Cathy Galle to this work and thank Darla Tharp for critical reading of the manuscript.

GRANTS
This work was supported by National Heart, Lung, and Blood Institute Grant HL-52490.

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