

ORIGINAL RESEARCH

Novel Mouse Model of Coronary Atherosclerosis With Myocardial Infarction: Insights Into Human CAD

Hong Chen¹, Qing Wan¹, Jianfeng Yang¹, Haojie Rao¹, Chuansheng Xu¹, Pengfei Xu, Xuejian Yang¹, Hongyue Wang¹, Wei Feng¹, Liqing Wang, Magnus Bäck¹, Robert E. Widdop¹, Feng Liu¹, Hong S. Lu¹, Alan Daugherty¹, Shengshou Hu¹, Garret A. FitzGerald¹, De-Pei Liu¹, Yu Huang¹, Weijun Jin, Miao Wang¹

BACKGROUND: Coronary artery disease is a chronic and multifactorial disease with acute manifestations. Little is known about the concomitant impact of hypercholesterolemia and hypertension on the development of coronary atherosclerosis.

METHODS: *ApoE* (apolipoprotein E) and *Scarb1* (scavenger receptor class B, type I), both associated with human hypercholesterolemia, were inactivated in mice by inserting a *Scarb1* knockdown cassette downstream of the *ApoE* promoter. Meanwhile, a doxycycline-inducible Ang II (angiotensin II) expression cassette was introduced. The resultant mutant mice (*ApoE^{SA/SA}*), isolated arteries, and pharmacological/genetic interventions were employed to assess the impacts of hypercholesterolemia and hypertension on coronary atherosclerosis and mechanisms.

RESULTS: *ApoE^{SA/SA}* mice developed mild coronary atherosclerosis with heart failure after chronic feeding with western diet. Strikingly, additional Ang II-induced hypertension, but not norepinephrine-induced hypertension, drastically accelerated coronary atherogenesis, exhibiting endothelial erosion, myeloid cell infiltration, spontaneous plaque rupture, and myocardial infarction, which was Ang II type 1 receptor-dependent. In contrast to this severe coronary atherosclerosis, femoral arteries were resistant to atherogenesis. Proteomic profiling revealed substantial differences in vasomotor reactivity and inflammation. Endothelium-dependent dilatation of coronary arteries was highly susceptible to the combination of hypercholesterolemia and hypertension compared with femoral arteries, and a similar vulnerability was also observed in human coronary arteries. Ex vivo exposure to Ang II markedly impaired endothelium-dependent dilatation in coronary arteries, but not in femoral arteries. Consistent with its less coronary atherogenic activity, norepinephrine dilated coronary arteries while constricting femoral arteries. Furthermore, dilatation of the coronary artery was more dependent on prostaglandins than that in femoral artery. Coronary prostaglandin biosynthesis was suppressed during atherogenesis and, conversely, an elevated coronary production of prostaglandins after methotrexate administration was associated with improved endothelial function and better cardiovascular survival.

CONCLUSIONS: The combination of hypercholesterolemia and Ang II-induced hypertension exerts strong synergistic effects on coronary atherogenesis. This is attributable to a selective vulnerability of coronary endothelium-dependent vasodilator responses to Ang II exposure and prostaglandin inhibition. *ApoE^{SA/SA}* represents a novel and convenient mouse model of coronary atherosclerosis with spontaneous myocardial infarction.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: angiotensin II ■ atherosclerosis ■ coronary artery disease ■ hypercholesterolemia ■ hypertension

Meet the First Author, see p 455

Correspondence to: Miao Wang, PhD, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, 167 Beilishi Rd, Xicheng District, Beijing 100037, China. Email miao.wang@pumc.edu.cn

*H. Chen, Q. Wan, J. Yang, and H. Rao contributed equally.

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Novelty and Significance

What Is Known?

- Coronary artery disease is a chronic and multifactorial disease with acute manifestations.
- Hypercholesterolemia and hypertension are main risk factors of coronary artery disease, and little is known about their concomitant impact on coronary atherosclerosis.
- Animal model of coronary atherosclerosis has been sought for a long time in the field.

What New Information Does This Article Contribute?

- The combination of hypercholesterolemia and angiotensin II–induced hypertension drastically accelerates coronary atherogenesis in mice, triggering spontaneous plaque rupture and myocardial infarction.
- Coronary arteries confer a selective vulnerability of endothelium-dependent vasodilator responses to angiotensin II exposure and prostaglandin inhibition.
- Human coronary arteries showed an increased endothelium vulnerability to hypercholesterolemia and hypertension relative to internal mammal arteries.

This study uncovers a strong synergy of hypercholesterolemia and hypertension in coronary atherogenesis, with destabilized plaques. Understanding the underlying mechanisms may afford opportunities for discovering new drugs for coronary artery disease, calling for an integrated approach to the formulation of the guidelines for the management of both plasma cholesterol and blood pressure. This study provides a novel mouse model of coronary atherosclerosis with spontaneous myocardial infarction—*ApoE^{SA/SA}* for coronary artery disease mechanistic research and drug discovery.

Nonstandard Abbreviations and Acronyms

Ang II	angiotensin II
AT1	angiotensin II type 1 receptor
CA	coronary artery
CAD	coronary artery disease
COX	cyclooxygenase
DEP	differentially expressed protein
Early AS	early-atherosclerotic mice
eNOS	endothelial NO synthase
FA	femoral artery
HDL	high-density lipoprotein
H-H	hypertension and hypercholesterolemia
ICAM1	intercellular cell adhesion molecule 1
IDL	intermediate-density lipoprotein
II	interleukin
Late AS	late-atherosclerotic mice
LDL-C	low-density lipoprotein cholesterol
MCP	monocyte chemotactic protein
ND	normal laboratory diet
PKA	protein kinase A
SCARB1	scavenger receptor class B, type I
Sdf-1	stromal cell–derived factor-1
VCAM1	vascular cell adhesion molecule 1
VLDL	very low-density lipoprotein
WD	western diet

Coronary artery disease (CAD), a leading cause of mortality and morbidity worldwide,¹ is a chronic and multifactorial disease with acute manifestations, particularly myocardial infarction. The primary cause of CAD is atherosclerosis.^{2,3} Prospective cohort studies have concluded that traditional cardiovascular risk factors, such as age, sex, family history, diet, obesity, increased LDL (low-density lipoprotein) cholesterol/APOB (apolipoprotein B)-containing lipoprotein cholesterol concentrations, and hypertension, contribute to atherogenesis and promote CAD development.^{4,5} Hypercholesterolemia and hypertension are 2 major modifiable risk factors of CAD. Intensive blood pressure lowering has been shown to reduce major adverse cardiovascular events in older patients with hypertension.⁶ However, this effect was not observed in an intermediate-risk population without cardiovascular disease,⁷ in contrast to the efficacy of reducing cholesterol concentrations.⁸ Therefore, hypercholesterolemia and hypertension might differentially contribute to the development of coronary atherosclerosis with potential interactions. Their concomitant impact on coronary atherogenesis remains unclear.

Mice deficient in genes associated with human hypercholesterolemia, such as genes for APOE or LDL receptor, develop atherosclerosis; however, few atherosclerotic lesions are detected in the coronary artery (CA),⁹ suggesting its complex biology. Mice deficient in both *ApoE* and *Scarb1* (scavenger receptor class B, type I, an HDL (high-density lipoprotein) receptor associated with hypercholesterolemia and CAD^{10,11}) develop coronary

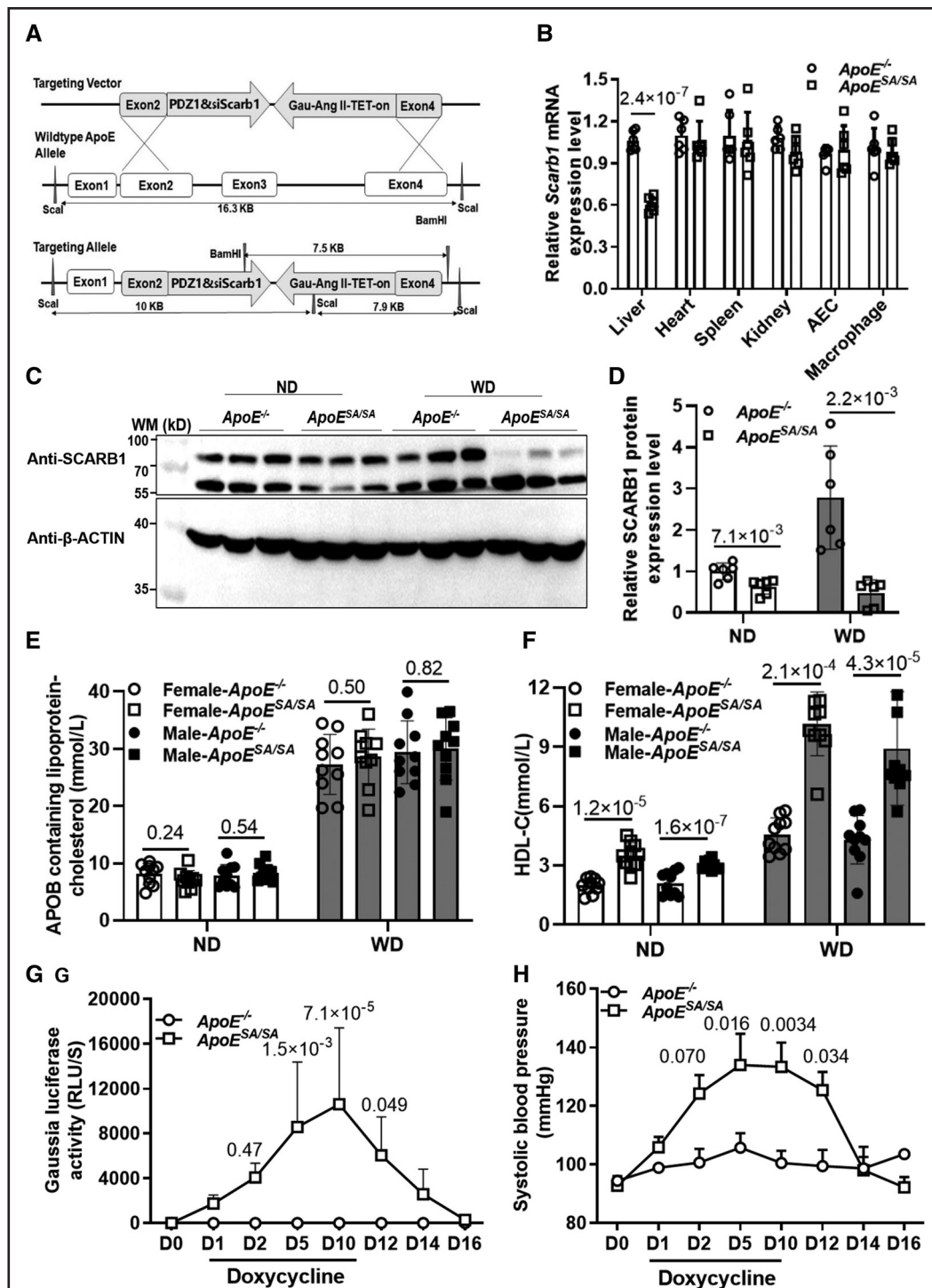


Figure 1. Inactivation of *ApoE* (apolipoprotein E) and *Scarb1* (scavenger receptor class B, type I) and inducible Ang II (angiotensin II)-mediated hypertension in mice.

A, Schematic illustration of the gene-targeting strategy at the *ApoE* locus to generate *ApoE^{SA/SA}* mice. Eight-week-old female *ApoE^{SA/SA}* or *ApoE^{-/-}* mice were used (**B** through **D**). **B**, Tissue abundance of *Scarb1* mRNA was detected using quantitative polymerase chain reaction, $n=6$ mice per group. **C**, Representative immunoblots of SCARB1 in liver. Similar results were observed in 2 independent experiments with 3 mice in each experiment ($n=6$ mice per group). **D**, Densitometry of SCARB1 protein abundance in liver. Eight-week-old *ApoE^{SA/SA}* or *ApoE^{-/-}* mice, including both males and females as indicated, were fed either a normal laboratory diet (ND) or western diet (WD) for 5 weeks (**E** and **F**). **E** and **F**, Plasma cholesterol concentrations of ApoB-containing lipoproteins (**E**) and high-density lipoprotein cholesterol (HDL-C; **F**) were measured in weaned mice, $n=10$ per group. Eight-week-old female *ApoE^{SA/SA}* or *ApoE^{-/-}* mice were fed ND with doxycycline (**G** and **H**). **G**, Plasma Gaussia luciferase activity was measured, $n=5$ per group. **H**, Systolic blood pressure was measured using a tail-cuff technique, $n=5$ per group. Student *t* test (**B**, **D** through **F**), and 2-way ANOVA with repeated measures (**G** and **H**) were used. AEC indicates aortic endothelial cell; PDZ1, postsynaptic density protein-95/discs large/zonula occludens-1 domain; RLU/s, relative light units/seconds; siScarb1, small interfering RNA for Scarb1; TET, tetracycline; and TET-on, tetracycline-inducible gene expression on type system.

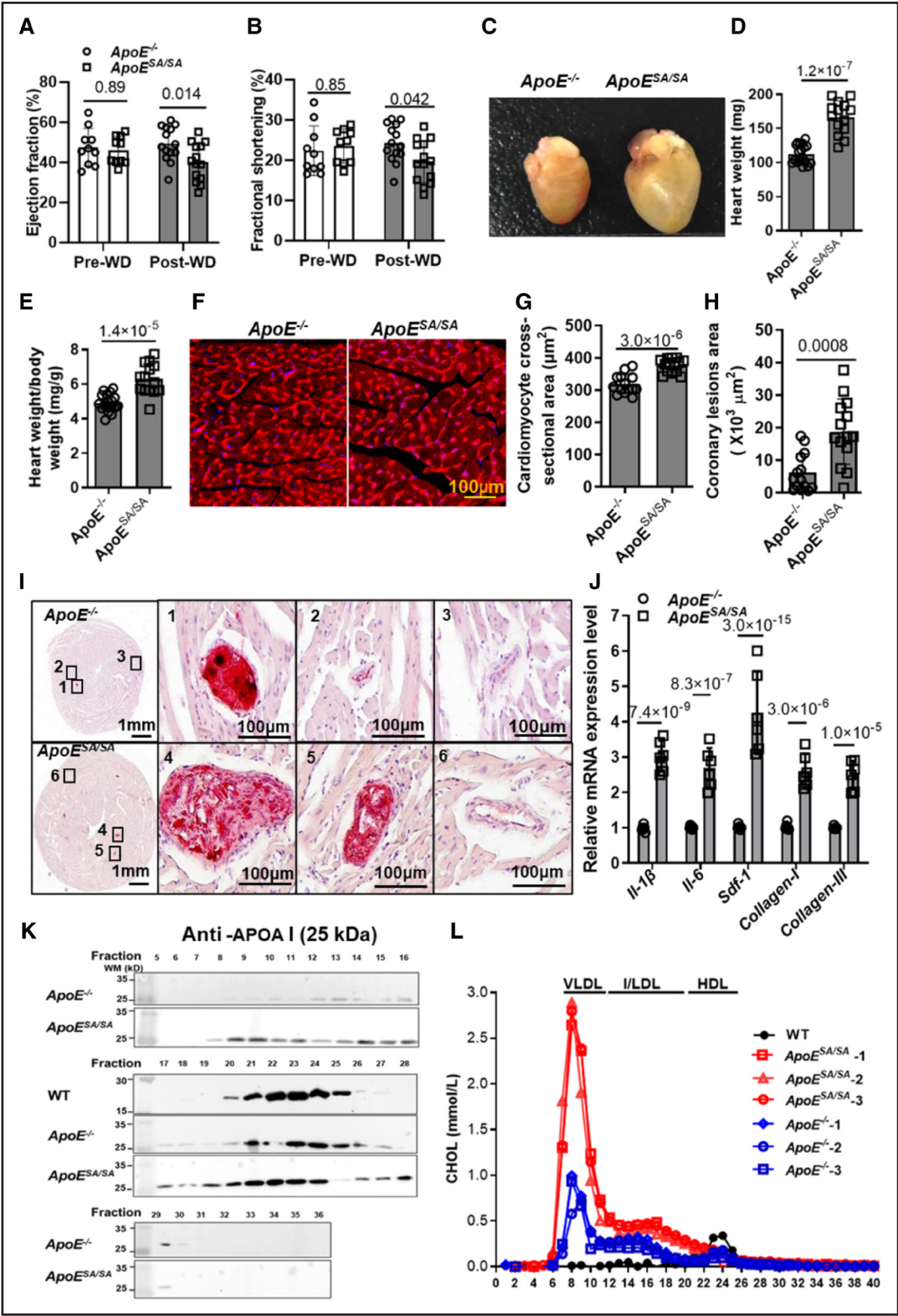


Figure 2. Hypercholesterolemia after *ApoE* and *Scarb1* inactivation resulted in chronic development of mild coronary atherosclerosis in mice.

Eight-week-old female *ApoE^{SA/SA}* or *ApoE^{-/-}* mice were fed a western diet (WD) for 16 weeks. Heart function, reflected by ejection fraction (**A**) and fractional shortening (**B**), was measured using echocardiography, before or after 16 weeks of WD feeding. Pre-WD group n=10, Post-WD group n=14. **C**, Representative images of heart weight. **D**, Quantification of heart weight to body weight, n=14. **E**, Ratio of heart weight to body weight, n=14. **F**, Images of myocardial sections stained with Alexa Flour 594-labeled wheat germ agglutinin (Bar=100 μm). **G**, Quantification of cardiomyocyte size (200 cells counted per heart, n=14). **H**, Quantification of coronary atherosclerotic lesion area, n=14. **I**, Representative Oil Red O stained myocardial sections (bar=1 mm) with magnified views of the boxed areas (bar=100 μm). **J**, Cardiac mRNA expression was (Continued)

atherosclerotic lesions, but also early death (at ≈ 5 weeks old),^{12,13} precluding their utility to assess the impact of chronic risk factors on coronary atherosclerosis. Coronary atherogenesis remains poorly understood. Despite the fact that lesion progression in coronary arteries is a more reliable predictor of clinical adverse events than that in the periphery,¹⁴ experimentally induced atherosclerotic plaques do not rupture,⁹ limiting our understanding of CAD symptoms.

The renin-angiotensin system plays a critical role in the pathophysiology of cardiovascular disease, and pharmacological inhibition of this hormonal system is the mainstay treatment for hypertension and chronic heart failure.¹⁵ The impact of the renin-angiotensin system activation on coronary atherogenesis is currently unknown, although Ang II (angiotensin II), the primary bioactive peptide of the renin-angiotensin system, augments aortic atherosclerosis in hypercholesterolemic mice^{16–19} and increases endothelial dysfunction.²⁰

We aimed to investigate the combined effects of hypercholesterolemia and Ang II-induced hypertension on coronary atherogenesis in mice. This study revealed a distinctive biology of the CA that makes it selectively vulnerable to atherogenesis, leading to advanced lesion formation and spontaneous rupture.

METHODS

Data Availability

The data supporting the findings of the study are available upon reasonable request from the corresponding authors. A detailed description of materials and methods is provided in the [Supplemental Material](#) and the [Major Resources Table](#).

RESULTS

Construction of a Mouse Model Deficient in *ApoE* and *Scarb1* and with Inducible Ang II Overproduction

To bypass the premature death in mice with global deficiency in *Scarb1* and *ApoE*, we took a strategy of *Scarb1* knockdown in a background of *ApoE* deficiency (Figure 1A): a *Scarb1* knockdown cassette, which consists of a coding sequence for a PDZ1 (postsynaptic density protein-95/discs large/zonula occludens-1) domain that blocks SCARB1 activity²¹ and a small interfering RNA for *Scarb1*, was inserted downstream and controlled by the endogenous *ApoE* promoter. The *ApoE* expression in

the resulting homozygote mutant mice (*ApoE*^{SA/SA}) was ablated (Figure S1). When *ApoE*^{SA/SA} mice were fed a normal laboratory diet (ND), the abundance of *Scarb1* mRNA showed no significant change in the heart, spleen, kidney, aortic endothelial cells, and macrophages (Figure 1B), but it was reduced in the liver to $\approx 60\%$ of that in *ApoE*^{-/-} mice (a commonly used mouse model of atherosclerosis, which exhibit no or little coronary atherosclerosis and have normal heart function). *Scarb1* expression was further reduced after feeding western diet (WD). This reduction was consistent with an increase in *ApoE* promoter activity in response to WD feeding (Figure S2), which enhanced the *ApoE* promoter-driven transcription of *Scarb1* knockdown cassette, increasing the efficiency of *Scarb1* depletion (*ApoE*^{-/-}; Figure 1C and 1D). The abundance of APOB-containing lipoprotein was similar between *ApoE*^{SA/SA} mice and *ApoE*^{-/-} mice (Figure 1E). As expected, *ApoE*^{SA/SA} mice had higher HDL cholesterol concentration than *ApoE*^{-/-} mice (Figure 1F; Table S1), indicating that hepatic SCARB1-mediated cholesterol uptake (reverse cholesterol transport) was blocked.

To introduce Ang II-induced hypertension, an Ang II overexpression cassette was placed downstream of the *Scarb1* knockdown element in the gene-targeting vector (Figure 1A), in which the Ang II coding sequence and its reporter gene (gaussian luciferase) were controlled by a Tet-on (tetracycline-inducible gene expression on type) system for doxycycline-inducible gene expression. Doxycycline administration via drinking water induced overproduction of Ang II and this overproduction correlated with gaussian luciferase activity (Figure S3). No luciferase activity was detected in the absence of doxycycline, indicating no spontaneous leakage of Ang II overproduction (Figure 1G). Accordingly, doxycycline administration induced hypertension in these mice and their blood pressure returned to baseline after its removal (Figure 1H).

Interaction of Hypercholesterolemia and Ang II-Induced Hypertension on Coronary Atherosclerosis

ApoE^{SA/SA} mice are fertile and develop normally (Video S1). No cardiac dysfunction was observed in 8-week WD-fed *ApoE*^{SA/SA} mice. After 16 weeks of WD feeding, *ApoE*^{SA/SA} mice exhibited a reduced ejection fraction and fractional shortening (Figure 2A and 2B), an increased left ventricle internal diameter (Figure S4), and enlarged heart and increased heart weight (Figure 2C through 2E).

Figure 2 Continued. assessed by quantitative polymerase chain reaction, $n=6$. **K**, Immunoblot analyses of Apo AI in the fractions shown in **L**. **L**, Plasma samples from individual animals were resolved by size exclusion fast protein liquid chromatography (FPLC), and total cholesterol concentrations of fractions were determined using an enzymatic assay. Two-way ANOVA with Sidak multiple comparisons test was used for statistical analysis (**A** and **B**). Student *t* test was used (**D** through **E**, **G**, **H**, and **J**). The images were selected based on the mean and are representative of ≥ 3 independent experiments throughout the studies. CHOL indicates cholesterol; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; and WT, wild type.

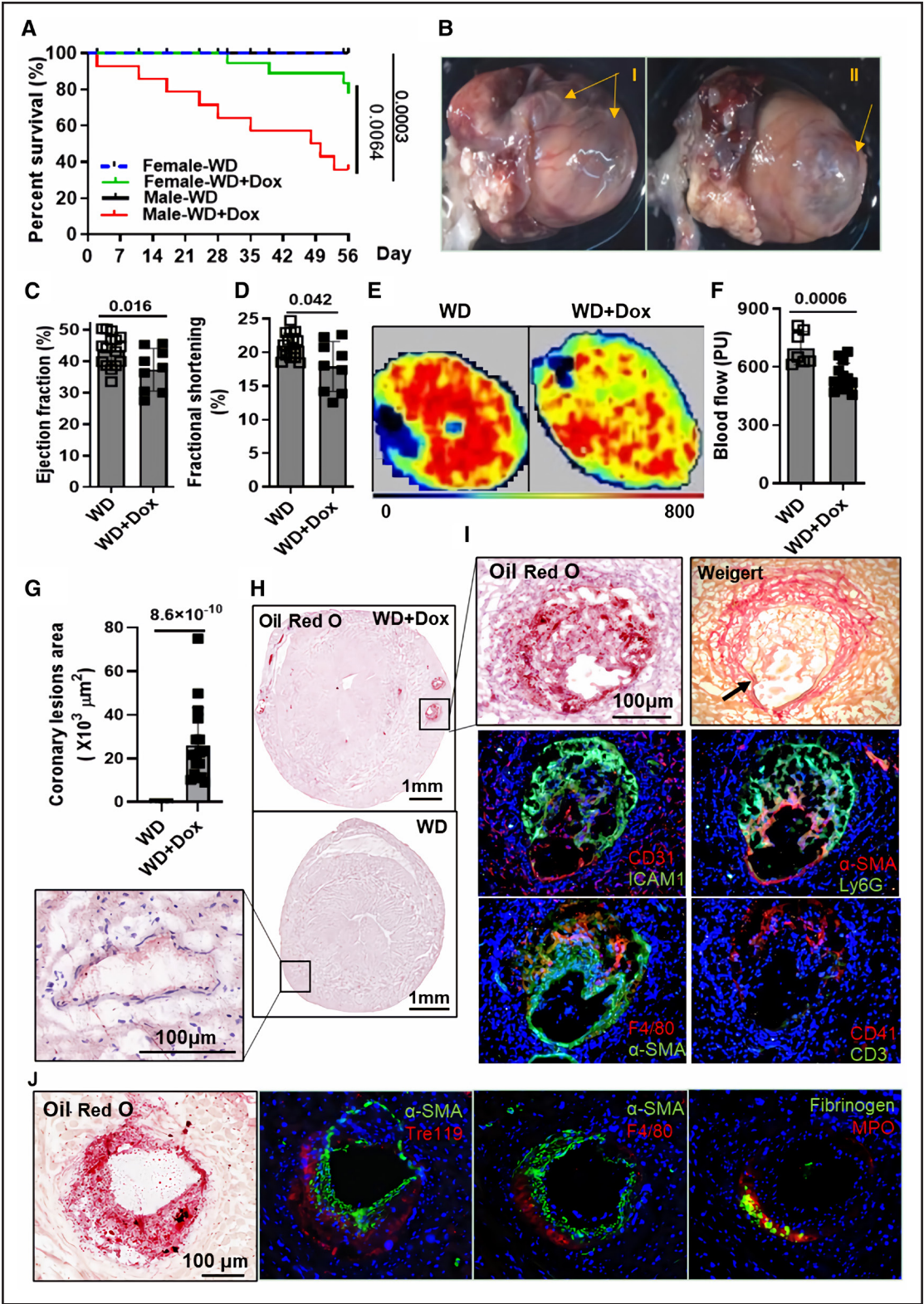


Figure 3. Synergistic effects of hypercholesterolemia and Ang II (angiotensin II)-induced hypertension on coronary atherogenesis with spontaneous plaque rupture.

Eight-week-old *ApoE*^{Sav/SA} mice were fed a western diet (WD) with and without doxycycline (Dox) administration. **A**, Kaplan-Meier survival curves were plotted for mice with 8 weeks of intervention (n=18 for each sex with WD, n=27 for each sex with WD+Dox). The Log-rank (Mantel-Cox) test, $P=3.0 \times 10^{-4}$, WD+Dox vs WD in males, $P=0.0064$, female vs male in WD+Dox. **B**, Autopsies were performed on mice that died during the intervention, revealing coronary atherosclerosis and myocardial infarction (I) and ventricular wall thinning/bulge after myocardial infarction (II), as marked by arrows. Echocardiographic quantification of ejection fraction (**C**) and fractional shortening (**D**) for male mice with 4 weeks of intervention, WD group: n=16, WD+Dox group: n=9. **E**, Representative images of cardiac blood perfusion examined by *(Continued)*

The size of cardiomyocytes was increased (Figure 2F and 2G). Oil Red O staining of the myocardium revealed lipid-rich atherosclerotic lesions that mainly affected small branches of coronary arteries and were distributed sparsely, with more lesions found in *ApoE^{SA/SA}* mice than in *ApoE^{-/-}* mice (18450 ± 12270 versus 6180 ± 3459 μm^2 ; Figure 2H and 2I; Figure S5). Cardiac mRNA abundance of inflammatory markers, Il (interleukin)-1 β , Il-6, Sdf-1 (stromal cell-derived factor-1), and of fibrosis-related products (collagen I and III) were upregulated in *ApoE^{SA/SA}* mice compared with *ApoE^{-/-}* mice, after 16 weeks WD feeding (Figure 2J).

To understand the dyslipidemia-induced coronary atherosclerosis in *ApoE^{SA/SA}* mice, plasma lipoproteins were resolved using the size exclusion fast protein liquid chromatography. A broad distribution of APOAI (a polipoprotein AI), the main protein component of HDL, was observed in the plasma fractions of *ApoE^{SA/SA}* and *ApoE^{-/-}* mice (Figure 2K). Compared with *ApoE^{-/-}* mice, *ApoE^{SA/SA}* mice exhibited significantly elevated cholesterol concentrations of VLDL (very low-density lipoprotein), IDL (intermediate-density lipoprotein)/LDL, and HDL (Figure 2L). *ApoE^{SA/SA}* mice also had a higher ratio of unesterified cholesterol to total cholesterol (Figure S6).

To investigate the effect of the concomitant presence of hypertension and hypercholesterolemia on coronary atherosclerosis, *ApoE^{SA/SA}* mice were fed a WD and administered doxycycline in drinking water. During the induction, sudden death was noted, with only 36% of males and 78% of females surviving for 8 weeks, whereas all WD-fed mice without doxycycline administration survived (Figure 3A; Video S2). Comorbidity included aortic rupture/aneurysm, kidney atrophy, and cerebral infarction (Figure S7). Notably, severe coronary atherosclerosis developed along with cardiac hypertrophy, myocardial infarction (ventricular wall thinning/bulge in some infarct regions), cardiac fibrosis, and elevation in plasma levels of cTnI (cardiac troponin I) and CKMB (creatine kinase MB; Figure 3B; Figure S8). Comparing *ApoE^{SA/SA}* mice administered WD and doxycycline for 4 weeks with those fed on WD alone, the concomitant presence of hypercholesterolemia and hypertension decreased cardiac function (Figure 3C and 3D), increased left ventricular anterior wall thickness (Figure S9), and reduced coronary blood flow (Figure 3E and 3F; Figure S10). Coronary atherosclerosis was significantly increased ($25\,952 \pm 1011$ versus 185 ± 16 μm^2 , ≈ 140 -fold increase) by 4-week

administration with WD+doxycycline versus with WD alone (Figure 3G and 3H). Histological analyses (Figure 3I) revealed that coronary atherosclerotic lesions in the *ApoE^{SA/SA}* mice were enriched in lipids and collagen deposition as well as elastic fiber breaks and were characterized by disrupted endothelial integrity/endothelial erosions (endothelial cells stained by CD31⁺). Smooth muscle cells (α -SMA⁺), neutrophils (Ly6G⁺), and macrophages (F4/80⁺), but not T lymphocytes (CD3⁺), were predominantly detected in these lesions. ICAM1 (intercellular cell adhesion molecule 1) was stained diffusely. Intralésional thrombosis was observed, as indicated by platelet staining (CD41⁺). Lesions with a large lipid core lacking a fibrous cap were observed frequently. Spontaneous plaque rupture was indicated by evident media tears, accompanied by red blood cell (Ter119⁺)/white blood cell (F4/80⁺, MPO⁺) infiltration and by the presence of blood clots (fibrinogen⁺; Figure 3J; Figure S11A and S11B). In particular, some lipid-positive occlusions had no vascular contour, reminiscent of distal occlusion resulting from proximate lesion rupture (Figure S11C).

We next determined the importance of Ang II or hypertension per se in the development of coronary atherosclerosis. Female *ApoE^{SA/SA}* mice were infused via mini osmotic pump with 100 ng/min norepinephrine, Ang II at 12.5 ng/min (low dose of Ang II that does not induce hypertension, Ang II-L) or 50 ng/min (high of Ang II dose that induces hypertension, Ang II-H),²² administered doxycycline, or doxycycline plus losartan (an antagonist of AT1 [Ang II type 1 receptor], 30 mg/kg/d), for 4 weeks on WD. During the 4 weeks, blood pressure remained high in the norepinephrine group, the Ang II-H group, and the doxycycline group (Figure 4A). In the Ang II-L group, blood pressure did not change. Losartan attenuated doxycycline-induced (ie, Ang II-mediated) hypertension. Mice in the Ang II-L group did not develop coronary atherosclerosis, but mice in the Ang II-H and doxycycline groups developed similarly severe coronary atherosclerosis (Figure 4B and 4C) with plaques particularly evident in the main branches of the coronary arterial tree. In contrast, norepinephrine administration did not result in coronary atherosclerosis as severe as Ang II-H or doxycycline administration, despite causing hypertension to a similar extent as seen in the Ang II-H group and doxycycline group. Losartan administration abolished doxycycline-induced coronary atherosclerosis. These results support the crucial role

Figure 3 Continued. I. laser doppler imaging. **F**, Quantification of cardiac blood flow, WD group: n=7, WD+Dox group: n=13. **G**, Quantification of coronary atherosclerotic lesions, n=17. **H**, Representative images of coronary atherosclerotic lesions stained by Oil Red O. Student *t* test (**C** and **D**, **F** and **G**). Male mice (**C** and **D**). Female mice (**E** through **G**). **I**, Representative images of coronary atherosclerotic lesions stained with Oil Red O, and Weigert (elastic fibers fiber: dark brown; collagen: red; arrow indicates broken elastic fibers) and for specific molecular markers as indicated in each panel. Images are representative of at least 3 independent experiments, with similar results obtained. **J**, Representative images of unstable plaques with signs of fission. Immune staining of specific markers is indicated in each part label. See text for details. The images selected are representative of 3 independent experiments. CD indicates cluster of differentiation; ICAM1, intercellular cell adhesion molecule 1; Ly6G, lymphocyte antigen 6 complex, locus G; MPO, myeloperoxidase; PU, perfusion unit; SMA, smooth muscle actin; and Ter-119, terminal erythroid marker (clone 119).

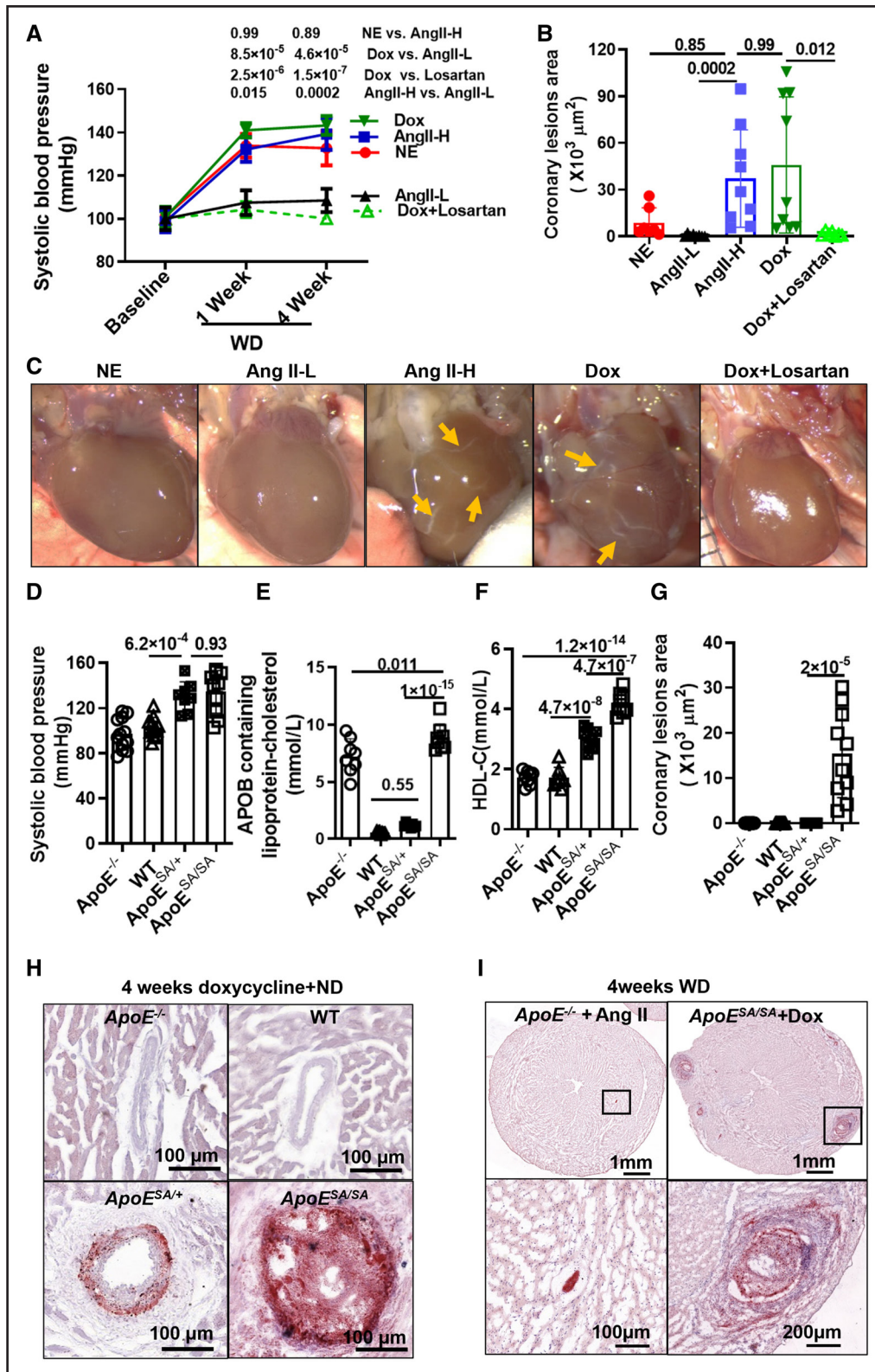


Figure 4. Selective susceptibility of coronary atherogenesis to Ang II (angiotensin II)-induced, but not norepinephrine-induced hypertension under hypercholesterolemia.

Eight-week-old female *ApoE^{SA/SA}* mice were infused with norepinephrine (NE), low (Ang II-L, 12.5 ng/min) or high (Ang II-H, 50 ng/min) dose of Ang II, or administered doxycycline (Dox) with or without losartan (30 mg/kg/d, by oral gavage) for 4 weeks (**A** through **C**). **A**, Blood pressure was measured at baseline, 1 and 4 weeks of administration, $n=7$ (for groups NE, Ang II-L, and Dox+losartan), $n=12$ (for groups Ang II-H and Dox). **B**, Quantification of coronary lesion area at 4-week infusion, $n=7$ (for groups NE, Ang II-L, and Dox+losartan), $n=9$ (for groups Ang II-H and Dox). **C**, Representative images of the heart where coronary occlusions are marked by arrows. Eight-week-old female mice of indicated genotype were administered doxycycline and normal laboratory diet for 4 weeks (**D** through **H**), $n=8$ (*ApoE^{SA/SA}* mice), $n=10$ (*ApoE^{-/-}*, WT, and (Continued)

of Ang II–AT1 receptor axis-dependent hypertension, rather than norepinephrine-induced hypertension, in coronary atherogenesis.

We further explored an interaction between hypercholesterolemia and Ang II-induced hypertension in coronary atherogenesis. Under doxycycline induction with ND feeding, *ApoE^{SA/+}* mice (heterozygous) exhibited hypertension comparable to *ApoE^{SA/SA}* mice, with similar concentrations of triglycerides, total cholesterol, and ApoB-containing lipoprotein cholesterol to wild-type mice and had higher HDL cholesterol concentrations than wild-type mice (Figure 4D through 4F; Table S2). This indicates that the single gene copy of Ang II inducible cassette was sufficiently functional. After doxycycline administration with ND feeding, *ApoE^{SA/SA}* mice became hypercholesterolemic and hypertensive and developed extensive coronary lesions within 4 weeks. In contrast, *ApoE^{SA/+}* mice were normocholesterolemic but hypertensive, and displayed coronary arterial thickening without apparent atherosclerotic lesions, whereas *ApoE^{-/-}* mice were hypercholesterolemic and normotensive, and showed no apparent coronary morphological abnormalities (Figure 4G and 4H). Although extensive coronary atherosclerosis developed after 4 weeks of hypercholesterolemia and hypertension, neither factor alone resulted in discernible coronary atherosclerotic lesions in *ApoE^{SA/SA}* mice. In addition, Ang II-infused *ApoE^{-/-}* mice barely developed coronary atherogenesis, in contrast to the exacerbated coronary atherosclerosis in the doxycycline-administered *ApoE^{SA/SA}* (Figure 4I), although the 2 groups of mice had similar levels of hypertension (Figure S12). Therefore, *Scarb1* suppression and Ang II induction were both required for *ApoE*-deficient mice to develop severe coronary atherosclerosis.

Differential Vasomotor Reactivity Underlies Coronary Atherosclerosis Susceptibility

High Athero-Susceptibility of Coronary Arteries Relative to Femoral Arteries and Their Distinctive Molecular Signatures

Interestingly, although markedly increased coronary atherogenesis was observed in the hypercholesterolemic and Ang II-induced hypertensive mice, few lesions were found in their femoral or saphenous arterial branches (Figure S13). The femoral arterial branches with diameter similar to the CA under basal physiology, developed media hyperplasia without obvious neointimal formation after WD plus doxycycline intervention, in sharp contrast

to the occlusive neointimal formation in coronary arteries (Figure 5A). Understanding these dichotomous atherogenic responses between the 2 vascular beds may reveal a mechanism for the high susceptibility of coronary arteries to atherosclerosis.

We next collected coronary and femoral arteries from *ApoE^{SA/SA}* mice fed ND (preatherosclerotic mice) or WD plus doxycycline administration (WD+doxycycline). These vessels were then subjected to proteomic analysis. A total of 2657 proteins were identified with certainty at a false discovery rate of 0.01. The samples were grouped into 6 clusters based on expression profiles: CA of preatherosclerotic mice, femoral artery (FA) of preatherosclerotic mice, CA of early atherosclerotic mice (3 days WD+doxycycline: early AS), FA of early AS, CA of late atherosclerotic mice (28 days WD+doxycycline, late AS), and FA of late AS (Figure 5B; Figure S14). The clusters formed were generally separated by vessel type. Notably, the CA clusters exhibited a further separation of the 3 stages of coronary atherogenesis (preatherosclerotic mice, early AS, and late AS), which was not observed within the FA clusters. In both early and late stages of atherogenesis, the number of differentially expressed proteins (DEPs) in CA was significantly higher than in FA (Figure 5C through 5I; Table S3). In early AS, the number of upregulated proteins was 93 in CA and only 6 in FA, whereas the number of downregulated proteins was 12 in FA and 2 in CA. In late AS, CA had 28 downregulated proteins compared with FA's 21 proteins, but upregulated proteins in CA were much more than in FA (364 versus 27), indicating a higher responsiveness of CA in atherogenesis than FA.

Multiple comparison and enrichment analyses did not detect enrichment for downregulated gene expression in early AS. In late AS, downregulated gene expression in CA was enriched in vascular contraction and activation of the renin-angiotensin system, whereas downregulated gene expression in FA was enriched in immunity/inflammation and lipid metabolism (Figure 5J). In response to hypercholesterolemia and hypertension, CA exhibited upregulated expression of genes for vascular remodeling, including extracellular matrix organization, peptide metabolic process, protein hydroxylation, and fiber organization during early and late stages of AS. The late stage of AS also showed upregulated immunity/inflammation (Figure 5J). These data suggest that the vasomotor reactivity might engage in coronary atherogenesis.

Figure 4 Continued. *ApoE^{SA/SA}* mice). **D**, Systolic blood pressure of mice after administration. **E** and **F**, Plasma concentrations of APOB-containing lipoproteins (**E**) and high-density lipoprotein cholesterol (HDL-C; **F**) were measured. **G**, Quantification of coronary lesion area. **H**, Representative Oil Red O staining of coronary arteries. Eight-week-old female *ApoE^{-/-}* mice were infused for 4 weeks with Ang II (50 ng/min) via osmotic mini-pump, and *ApoE^{SA/SA}* mice were administered Dox for 4 weeks. **I**, Representative Oil Red O stained myocardial sections. Tissue staining data are representative of 3 independent experiments. Two-way ANOVA with repeated measures (**A**) and 1-way ANOVA with Newman-Keuls multiple comparisons post hoc test (**B**, **D** through **G**) were used for statistical analysis. WD indicates western diet.

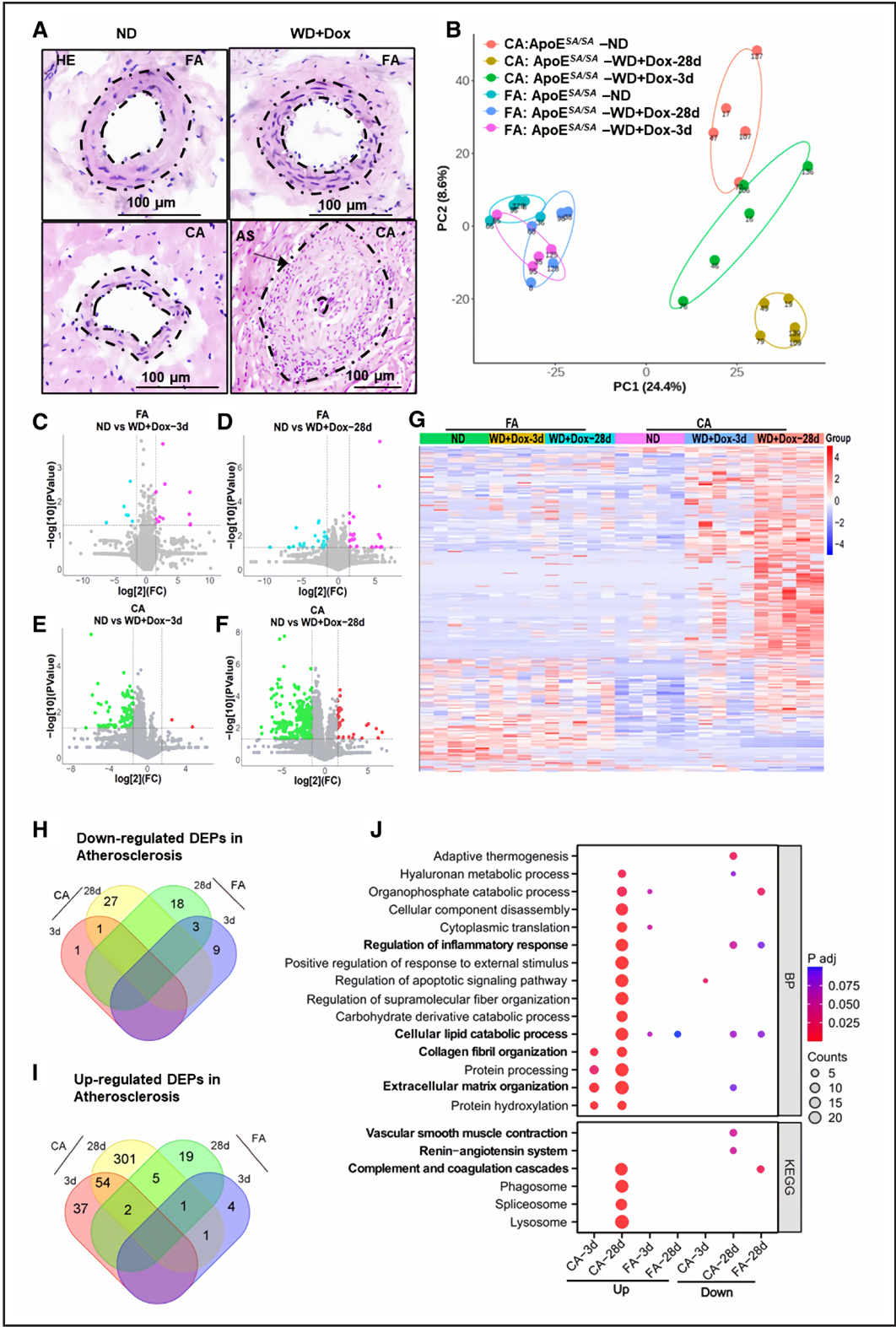


Figure 5. High athero-susceptibility of coronary arteries relative to femoral arteries and their distinctive molecular signatures in vasomotor reactivity and inflammation.

Coronary artery (CA) and femoral artery (FA) were collected from 8-week-old male ApoE^{SA/SA} mice fed normal laboratory diet (ND, preatherosclerotic mice) or western diet (WD) plus doxycycline administration (WD+ doxycycline [Dox]) for 3 days (WD+Dox-3d) or 28 days (WD+Dox-28d). **A**, Representative images of HE (Hematoxylin-Eosin)-stained cross-sections of femoral and coronary arteries from ApoE^{SA/SA} mice fed either ND or WD+Dox for up to 28 days. Tissue staining data are representative of 3 independent experiments. CA and FA vessels were subjected to proteomics analyses. **B**, Principal component (PC) analysis 2-dimensional scatter plot indicates differential protein expression patterns of coronary and femoral arteries isolated from ApoE^{SA/SA} mice. Splashes represent different samples. Numbers (*Continued*)

Selective Impairment of Coronary Vasomotor Reaction Under Hypercholesterolemia and Hypertension

Next, vasomotor reactivity 3 days after induction of hypercholesterolemia and hypertension was examined *ex vivo* in coronary and femoral arteries. Mice were fed either ND or WD plus doxycycline administration for 3 days. Coronary and femoral arteries of similar diameter (180–200 μ m) were isolated and their vascular contraction and relaxation were compared using wire myography. The contraction to potassium chloride (KCl) or a thromboxane receptor agonist (U46619) in both coronary and femoral arteries was similar between *ApoE^{SA/SA}* and *ApoE^{-/-}* mice fed ND. When hypertension was induced in *ApoE^{SA/SA}* mice, coronary arteries, but not femoral arteries, exhibited a significant decrease in their contractile responses (Figure 6A and 6B). Moreover, the dilator response of coronary arteries to acetylcholine was attenuated in *ApoE^{SA/SA}* mice compared with *ApoE^{-/-}* mice, and this endothelium-dependent relaxation was absent 3 days after induction of hypercholesterolemia and hypertension (Figure 6C). In contrast to the coronary response, femoral arteries did not show endothelial dysfunction (Figure 6D). The endothelium-independent relaxations induced by sodium nitroprusside were similar in both types of arteries (Figure 6E). Consistent with the early coronary endothelial dysfunction, Oil Red O staining showed an increase in vascular lipid deposition after the dual atherogenic insults (Figure S15).

We further compared the vasomotor reactivity of human coronary arteries and internal mammary arteries. The contraction to U46619 was reduced significantly in coronary arteries from patients with both hypertension and hypercholesterolemia (H-H), as compared with those from subjects without H-H (control). In contrast, internal mammary arteries from patients with H-H retained U46619-induced contraction (Figure 6F). Conversely, coronary arteries from patients with H-H did not relax in response to acetylcholine, whereas internal mammary arteries from patients with H-H still exhibited endothelium-dependent relaxations (Figure 6G). Again, the endothelium-independent relaxations induced by sodium nitroprusside were similar in both arteries (Figure 6H). These observations on human vascular function

also support a high vulnerability of coronary endothelial function to hypercholesterolemia and hypertension.

Distinctive Regulation of Coronary Vasomotor Reactivity by Ang II, β -Adrenergic Receptors, and Prostaglandins

To further explore the molecular basis of the differential athero-susceptibility of coronary arteries, coronary and femoral arteries were isolated from wild-type mice and compared for vascular contraction and relaxation. Both arteries contracted in response to KCl and U46619 (Figure 7A). Femoral arteries contracted in response to Ang II and norepinephrine, while coronary arteries did not. Instead, norepinephrine, acetylcholine, and isoproterenol, produced relaxations in U46619 precontracted coronary arteries (Figure 7B). The incubation of Ang II eliminated the acetylcholine-induced endothelium-dependent relaxation of coronary arteries, but not in femoral arteries (Figure 7C). Thus, Ang II specifically modifies endothelium-dependent relaxations in coronary arteries. The distinction in vasomotor response to norepinephrine/isoproterenol between coronary arteries and femoral arteries reflects their differential signaling of adrenergic receptor: that is, β -adrenergic receptor dominantly mediated coronary vasodilation (Figure 7D and 7E), whereas α -adrenergic receptor-mediated femoral contraction that was also subjected to opposite modulation by β -adrenergic receptor signaling (Figure 7F).

β -Adrenergic receptors are known to couple with cAMP-PKA (protein kinase A) signaling pathway or with G_i /nitric oxide (NO) pathway in the heart.²³ We next compared roles of NO and prostaglandins (which regulate cAMP-PKA signaling) in vasodilation between coronary and femoral arteries. Administration with a NO synthase inhibitor NG-nitro-L-arginine methyl ester inhibited acetylcholine-induced relaxations in both coronary and femoral arteries of wild-type mice (Figure 7G). Treatment with SC560, an inhibitor of the constitutively expressed prostaglandin-synthesizing enzyme COX (cyclooxygenase)-1, suppressed acetylcholine-induced relaxation in coronary arteries but not in femoral arteries (Figure 7H). In addition, coronary arteries from *Cox-1* deficient mice also exhibited reduced acetylcholine-induced

Figure 5 Continued. represent sample ID. Integrative analysis of the proteome of CA and FA shows differentially expressed proteins (DEPs, defined as $\log_2$ (fold change [FC]) > 1.5 or < -1.5 , and $P < 0.05$, statistical analysis of the identified proteins was done by using Perseus_2.0.3.1). **C**, Volcano plot of DEPs of FA between preatherosclerotic and early-atherosclerotic mice (WD+Dox-3d). **D**, Volcano plot of DEPs of FA between preatherosclerotic and late-atherosclerotic mice (WD+Dox-28d). **E**, Volcano plot of DEPs of CA between preatherosclerotic and early-atherosclerotic mice (WD+Dox-3d). **F**, Volcano plot of DEPs of CA between preatherosclerotic and late-atherosclerotic mice (WD+Dox-28d). Splashes represent different proteins. Green, red, blue, and purple splashes represent proteins with significantly different expression. Gray splashes represent proteins without significantly different expression. The DEPs were used for further analysis (**G** through **K**). **G**, Heat map and hierarchical clustering for DEPs of CA and FA, with differential abundances defined by the blue-to-red color table Z scores calculated from row-wise normalization. **H**, Venn diagram of downregulated DEPs of CA and FA in atherosclerosis. **I**, Venn diagram of upregulated DEPs of CA and FA in atherosclerosis. **J**, Dot plot for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment for DEPs of CA and FA in atherosclerosis. Dot plot was generated using an online tool (<https://www.helixlife.cn/>). DEPs: Differentially expressed proteins. Volcano plot and Venn diagram were generated using Bioladder (<https://www.bioladder.cn/>). 3d denotes mice were administrated WD plus doxycycline for 3 days, and 28d denotes mice were administrated WD plus doxycycline for 28 days. $n=5$, male *ApoE^{SA/SA}* mice. AS indicates atherosclerosis; BP, blood pressure; and P adj, P value adjusted (by Benjamini-Hochberg).

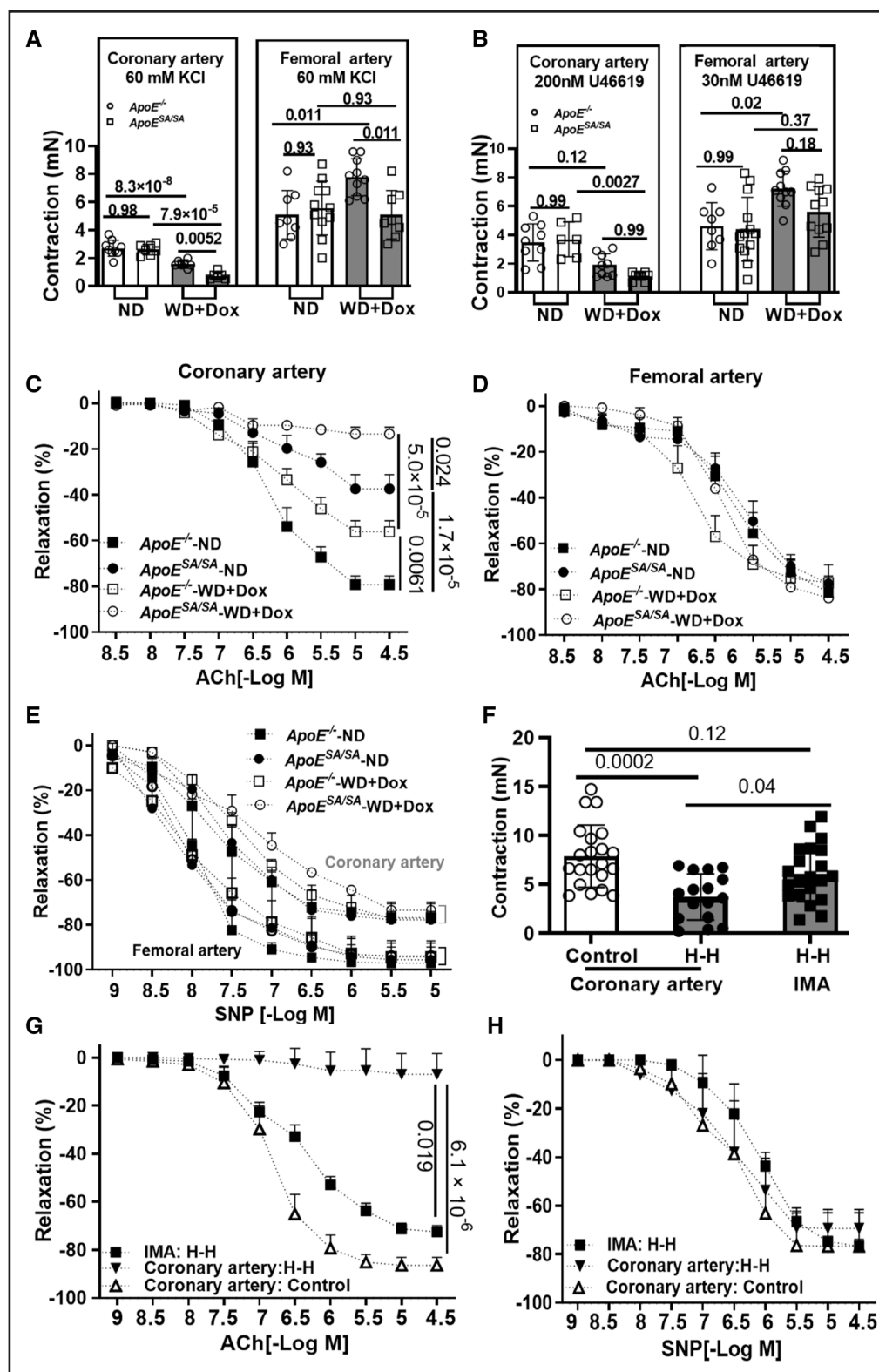


Figure 6. Selective impairment of coronary vasomotor reaction under hypercholesterolemia and hypertension.

Eight-week-old male $ApoE^{-/-}$ and $ApoE^{-/-}SA/SA$ mice were initiated with either normal laboratory diet (ND), or western diet (WD) with doxycycline (Dox), for 3 days, $n=9$ each group. Vascular contraction to potassium chloride (KCl; A) and U46619 (B) at the indicated concentrations were measured for isolated coronary and femoral arteries. Values are mean $\pm$ SEM. $n=9$, 6, 9, 7 coronary arteries for the groups as labeled from left to right (same way of sample size presentation applies to other similarly labeled groups); $n=8$, 13, 10, 11 femoral arteries for the groups as labeled from left to right. Concentration-response curve to acetylcholine (ACh) for coronary arteries (C) and femoral arteries (D). Relaxation was expressed as the percentage of contraction in response to U46619. $n=7$, 6, 6, 7 coronary arteries; $n=8$, 12, 9, and 10 femoral arteries. Concentration-response curve to sodium nitroprusside (SNP) for coronary arteries and femoral arteries (E). $n=4$, 4, 5, 5 coronary (Continued)

relaxations (Figure S16). Treatment with celecoxib, an inhibitor of the inducible isoform COX-2 did not suppress acetylcholine-induced relaxation (Figure S17), likely due to low expression levels of COX-2 under physiological conditions.

Prostaglandin concentrations in the coronary perfusate were measured in the hearts isolated from *ApoE^{SA/SA}* mice fed a WD and administered doxycycline, as well as from those fed ND. The induction of hypercholesterolemia and hypertension resulted in a reduction of PGE₂ (prostaglandin E₂) and 6-keto-PGF_{1 α} (prostaglandin F_{1 α}) (the stable hydrolysis product of PGI₂ [prostacyclin]; Figure 8A and 8B). This suggests that suppression of these vasodilator prostaglandins might contribute to the impaired coronary endothelial function (Figure 6C). Prostaglandins have a very short half-life and multiple receptors. Methotrexate, an immunosuppressant that induces COX-2 expression,^{24,25} was used to interrogate the role of prostaglandins in CA function in *ApoE^{SA/SA}* mice. Methotrexate significantly improved survival rates of hypercholesterolemic and hypertensive mice (Figure 8C), without significant changing coronary lesion size in surviving mice (Figure S18). It did not affect blood pressure or plasma lipid concentrations (Figure S19A through S19C); additionally, it reduced systemic inflammation as evidenced by the decreased plasma concentrations of IL-1 β , MCP (monocyte chemoattractant protein)-5, P-selectin, VCAM1 (vascular cell adhesion molecule 1), and ICAM1 (Figure S19D through S19H). To investigate early vascular changes and the potential involvement of prostaglandins, *ApoE^{SA/SA}* mice were fed WD and administered doxycycline. They were then randomly assigned to receive intraperitoneal injection of vehicle, methotrexate, or methotrexate plus celecoxib for 3 days. The methotrexate group showed less early lipid deposition in the CA compared with the vehicle group (Figure 8D and 8E). Meanwhile, methotrexate induced coronary production of PGE₂ and PGI₂ (Figure 8F and 8G), consistent with the upregulated expression of COX-2 in the CA (Figure 8H). With an unaltered CA contraction (Figure 8I), methotrexate improved endothelial function, and this effect was substantially attenuated by cotreatment with the COX-2 inhibitor celecoxib, albeit COX-1 or other effects of methotrexate might still contribute to the vasodilatation (Figure 8J).

DISCUSSION

Global knockout of both *ApoE* and *Scarb1* resulted in coronary complications with premature death (all mice

died at $\approx$ 5 weeks old),^{12,13} precluding its use in assessing the effects of chronic risk factors on coronary atherosclerosis. By knocking down *Scarb1* predominantly in the liver, where reverse cholesterol transport occurs, we were able to bypass the premature death to assess interactions of hypercholesterolemia and hypertension on coronary atherosclerosis. Strikingly, this study uncovers a strong synergistic impact of hypercholesterolemia and Ang II-mediated hypertension on the development of coronary atherosclerosis, featuring many of the key features of advanced CAD in humans. These include multiple-vessel atherosclerotic occlusion along the coronary arterial tree, unstable plaques (exemplified by endothelium erosion, myeloid cell inflammation, plaque fissure, intralumenal thrombosis, and distal blockade), myocardial infarction, and heart failure. Such severe coronary atherosclerosis did not occur with either hypercholesterolemia or hypertension alone.

Insights into mechanisms underlying coronary atherogenesis were gained by proteomic profiling and comparing coronary arteries and femoral arteries, the 2 vascular beds exhibiting distinctive athero-susceptibility. Coronary arteries were highly metabolically active during vascular remodeling. They responded readily to hypercholesterolemia and hypertension by expressing more proteins in structural transformation at the onset of atherosclerosis and by expressing fewer proteins in vascular contraction and more proteins in immune response/inflammation at advanced stages of atherosclerosis. In contrast, femoral arteries exhibited downregulation of lipid metabolism and immune response after atherogenic insults, while maintaining their resilient structure as major conduit arteries. The molecular signature suggests potential importance of vasomotor responses in dictating coronary arterial susceptibility to vascular inflammation and atherogenesis. In fact, when hypercholesterolemia was exacerbated, coronary arterial endothelium function became particularly vulnerable and was essentially abolished shortly after Ang II-mediated hypertension was superimposed to hypercholesterolemia, while femoral arterial endothelium-dependent relaxation remained. It is worth noting that human autopsy findings indicate that atherosclerosis progressed more rapidly in coronary arteries than in femoral arteries.²⁶

This study reveals critical roles of Ang II and prostaglandins in the coronary vasomotor reaction underlying coronary atherogenesis. Hemodynamic stress is critical in atherogenesis. Here, hypertension induced by Ang II, but not by norepinephrine, drastically accelerated

Figure 6 Continued. arteries; n=7, 6, 7, 6 femoral arteries. Human coronary arteries (CA) and internal mammary arteries (IMA) from patients with both hypertension and hypercholesterolemia (H-H) and from those without H-H (Control) were assessed for vasoconstriction response to 30 nmol/L U46619 (F). n=13, 10, 9 patients. Values are mean $\pm$ SEM. n=21, 16, 21 vessels. Concentration-response curves to ACh (G) and SNP (H) in human CA and IMA. Vessels were precontracted with U46619. G, n=13, 9, 12 vessels. H, n=6, 6, 7 vessels. One-way ANOVA with Tukey multiple comparisons test was used for statistical analysis (A, B, and F). Cumulative concentration-response curves (C through E, H) were analyzed by comparing area under the curve with 1-way ANOVA with Tukey multiple comparisons test.

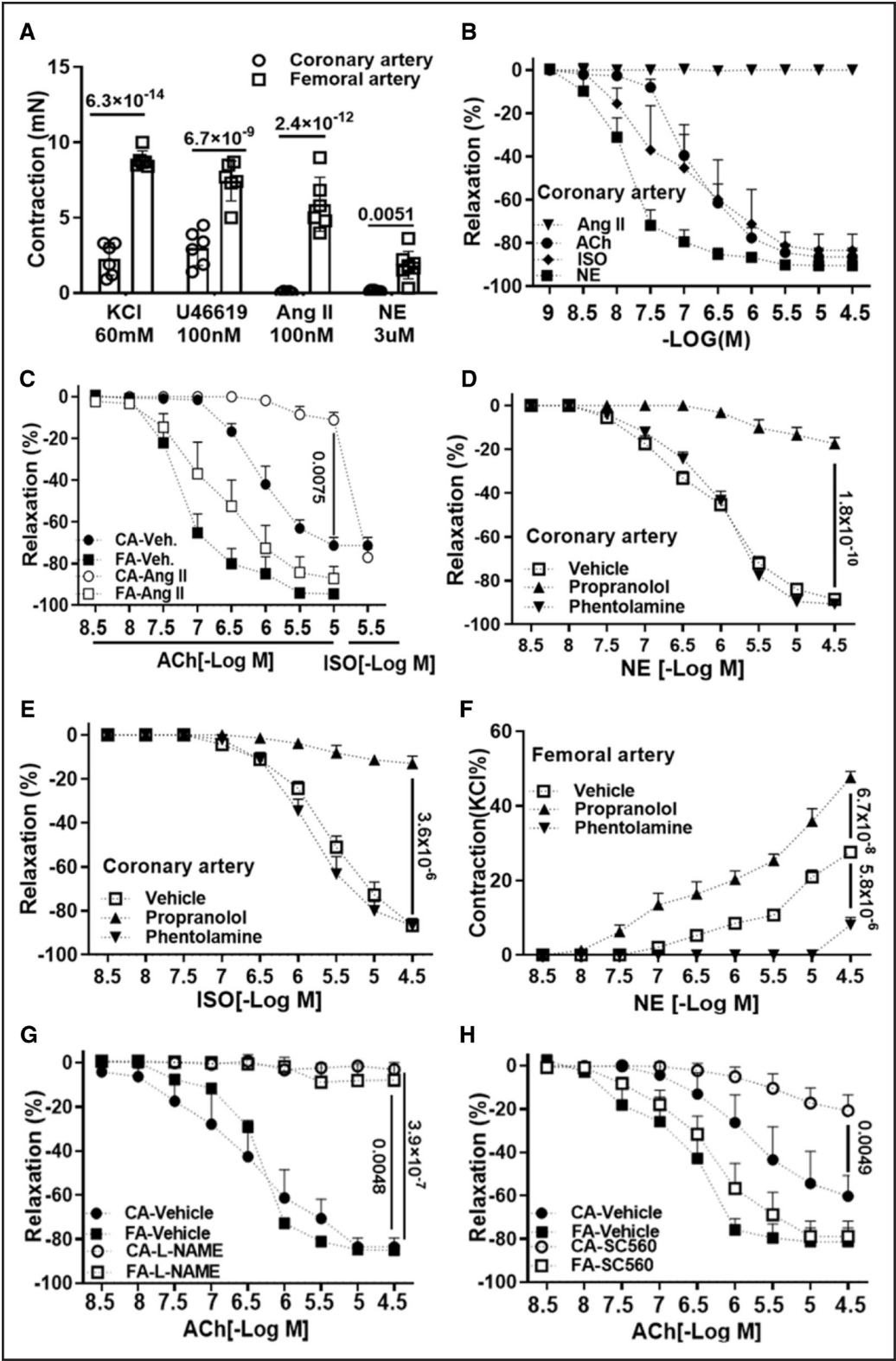


Figure 7. Distinctive regulation of vasomotor reactivities by Ang II (angiotensin II), norepinephrine, and prostaglandins in coronary arteries (CA) compared with femoral arteries (FA). Signaling pathways of vasorelaxation were characterized using CA and FA isolated from 8-week-old male wild-type mice fed normal laboratory diet. Vascular contraction to potassium chloride (KCl), U46619, Ang II, and norepinephrine (NE), at indicated concentrations, were determined for coronary and femoral arteries (A). n=6, 6, 6, 8. Student t test was used for statistical analysis. B, Relaxation-response curve to Ang II, acetylcholine (ACh), isoproterenol (ISO), and NE in coronary arteries, n=6, 7, 6, 6. C, Concentration-response curve to ACh in arteries precontracted with U46619. Ang II (100 nmol/L) significantly inhibited the coronary relaxation responses to ACh (not ISO, 3 μM), (Continued)

coronary atherogenesis, despite their induction of similar degree of systemic hypertension. Ang II constricted peripheral arteries and inhibited endothelium-dependent relaxation of coronary arteries, in contrast, norepinephrine dilated coronary arteries (β -receptor mediated) while constricting peripheral arteries (α -receptor mediated). The differential effects of Ang II and norepinephrine on coronary dilatation and atherogenesis support the notion that impairment of coronary dilatation by Ang II increases local hemodynamic stress on the vessel wall, promoting atherosclerosis, and increased coronary vasodilatation/vascular compliance upon hemodynamic stimuli protects against coronary atherogenesis. It is also suggested that hypertension of different cause may unequally predispose coronary atherogenesis and confer varied risk of CAD.

In our study, losartan treatment reversed Ang II-induced hypertension, endothelial dysfunction, and coronary atherosclerosis, suggesting that targeting the Ang II–AT1 receptor axis at the coronary endothelium may represent an effective strategy for preventing CAD in patients with both hypercholesterolemia and hypertension. Ang II impairs coronary endothelium-dependent NO-mediated dilation via AT1 receptor activation of NADPH oxidase (nicotinamide adenine dinucleotide phosphate oxidase) and superoxide release.²⁷ Ang II at a lower infusion rate without increasing blood pressure, did not significantly increase coronary atherosclerosis. This reinforces the importance of the hyperlipidemia-hypertension interaction through the Ang II–AT1 receptor activation in coronary atherosclerosis.

Prostaglandins play an important role in coronary vascular function. PGE₂-EP4 signaling constrains coronary ischemia-reperfusion injury, consistent with a suppressing effect of PGE₂ on endothelial dysfunction and neutrophil activation.²⁸ Prostacyclin 2 interacts with NO signaling via activating eNOS (endothelial NO synthase) signaling, which causes vascular smooth muscle relaxation.²⁹ Methotrexate-induced synthesis of coronary prostacyclin 2/E₂ may preserve coronary endothelium function, preventing atherogenesis and improving lesion stability. The use of non-steroidal anti-inflammatory drugs (NSAIDs) was associated with an increased risk of coronary events, such as cardiovascular death and nonfatal myocardial infarction, but not with cerebral vascular events like stroke.³⁰ Furthermore, administration of indomethacin caused CA constriction in patients with CAD.³¹ These clinical observations also support the importance of prostaglandins in coronary

functionality, consistent with our experimental study. Given multiplicity of prostanoids and their receptors, functions of individual prostanoid signaling await future study.

In summary, the combination of hypercholesterolemia and Ang II-induced hypertension drastically accelerated coronary atherogenesis in mice, resulting in a spectrum of CAD that recapitulates many features of human biology. The Ang II–AT1 receptor axis and prostaglandin are key in modulating coronary endothelium-dependent vasodilation that underlies the selective vulnerability of coronary atherogenesis. A schematic illustration of the selective susceptibility of coronary arteries to hypercholesterolemia and hypertension and its mechanistic basis is shown (Figure S20).

This study also provides a novel mouse model of coronary atherosclerosis. *ApoE^{SA/SA}* mice develop normal and are fertile. Diet-modifiable hypercholesterolemia led to chronic coronary atherosclerosis in these mice, and additional hypertension resulted in advanced coronary atherosclerotic plaques with spontaneous rupture and myocardial infarction. This mouse model is useful in elucidating CAD mechanisms and has an advantage in assessing multiple (chronic) risk factors and their interactions and pharmacological interventions for CAD. *ApoE^{SA/SA}* mice carry only 1 genetic mutant locus, rendering it easy to breed and cross with other strains.

Although separate guidelines are available for managing blood cholesterol concentrations and blood pressure, with targeted values changed over time, hyperlipidemia and hypertension often coexist. This study raises a concept that when hypercholesterolemia reaches certain extent, or results from specific genetic traits, or fails to meet targeted value after treatment, superimposed hypertension of certain cause could be detrimental to coronary atherogenesis. Their interaction highlights the necessity of developing a more integrated approach to formulating such guidelines.

LIMITATIONS

This experimental study reveals a strong interaction between hypercholesterolemia and hypertension in coronary atherogenesis. Extrapolation of these animal observations to clinical settings in subjects with different extents/types of hypercholesterolemia and hypertension and individualized intervention await future clinical investigation.

Figure 7 Continued. but it did not influence femoral artery relaxation responses to ACh, n=6. Relaxation-response curve to NE (D) or ISO (E) in CA, n=6. Contraction-response curve to NE (F) in FA, n=6. Propranolol (β -adrenergic receptor antagonist) and phentolamine (α -adrenergic receptor antagonist) were incubated at a concentration of 10 μ M. NG-nitro-L-arginine methyl ester (100 μ M) significantly inhibited both CA and FA relaxation responses to ACh (G), n=6. SC560 (5 μ M) inhibited coronary (not femoral) artery relaxation responses to ACh (H), n=10 CA, n=8 FA. Cumulative concentration-response curves (A through H) were analyzed by comparing area under the curve with 1-way ANOVA with Tukey multiple comparisons test. Veh. indicates vehicle.

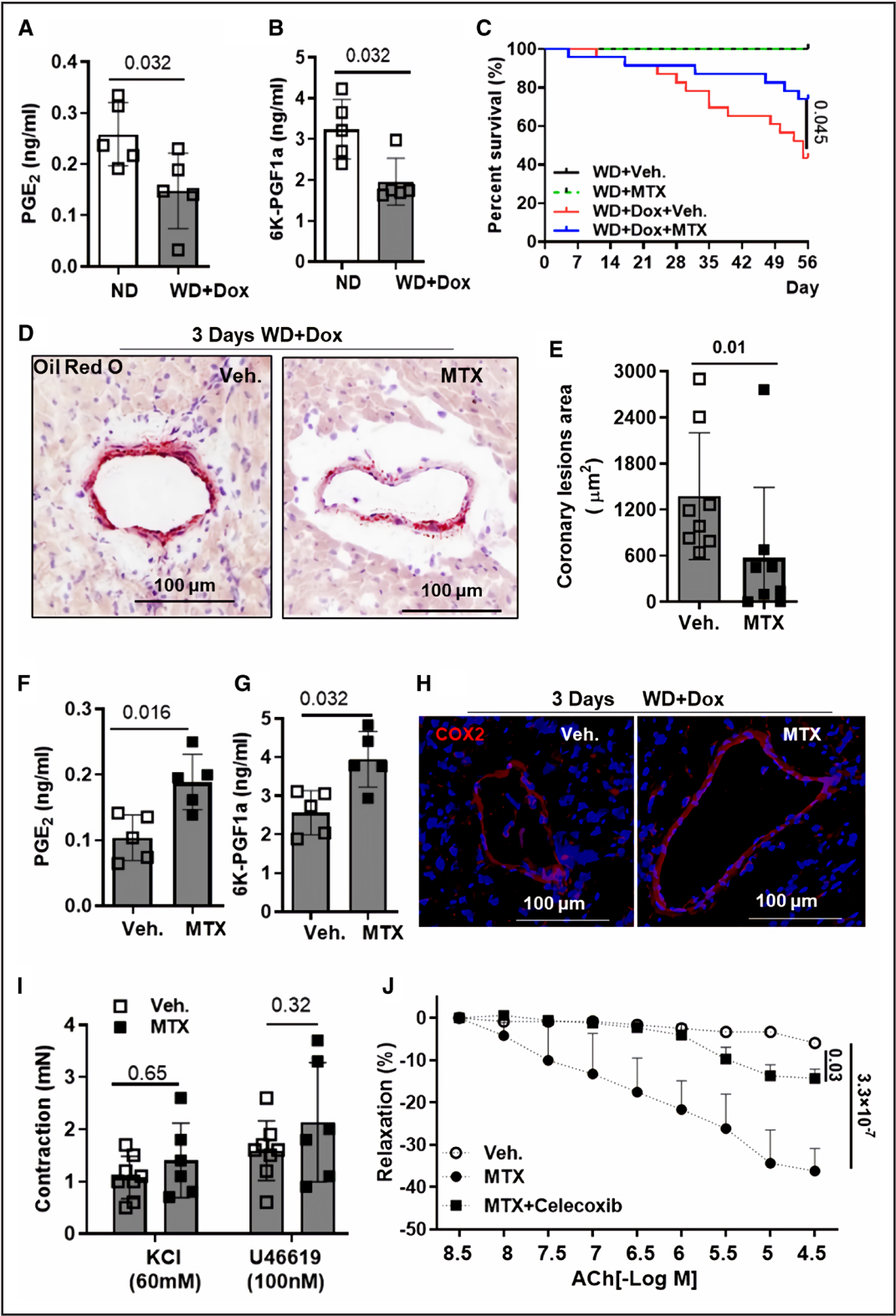


Figure 8. Coronary prostaglandin production was suppressed in atherogenesis and its upregulation by methotrexate associated with cardiovascular protection.

To examine coronary prostaglandin biosynthesis, hearts were isolated from male *ApoE*^{SA/SA} mice fed normal laboratory diet (ND), or western diet (WD) plus doxycycline (Dox; 4 weeks). The hearts were perfused in a modified Langendorff system. Concentrations of PGE₂ (A) and 6k-PGF_{1α} (B) in the effluent of ex vivo perfused hearts were determined by HPLC-MS-MS. n=5. Eight weeks old male *ApoE*^{SA/SA} mice were administered WD with Dox and randomized to receive vehicle (Veh.) /saline (WD+Dox+Veh) or methotrexate (WD+Dox+MTX) for 8 weeks. n=23. A separate set of *ApoE*^{SA/SA} mice fed WD and administered with MTX (WD+MTX) or vehicle (WD+Veh) were used as (Continued)

Figure 8 Continued. control. $n=12$. These control mice developed no apparent atherosclerotic lesion in the short study period. **C**, Kaplan-Meier survival curve, Log-rank (Mantel-Cox) Test. **D**, Representative images of lipid deposition in coronary arteries stained by Oil Red O. **E**, Quantification of coronary atherosclerotic lesions in mice with 3 days intervention, $n=8$ mice. Concentrations of PGE_2 (**F**) and $6\text{k-PGF}_{1\alpha}$ (**G**) in the effluent of ex vivo perfused hearts were determined by HPLC-MS-MS. $n=5$. **H**, Representative histology staining of COX-2 (cyclooxygenase 2) in coronary arteries. **I**, Mouse coronary artery constriction to KCl (60 mmol/L) and U46619 (100 nmol/L). $n=8$, 6 vessels. **J**, Concentration-relaxation response to acetylcholine (ACh) of isolated mouse coronary arteries. Relaxation is expressed as a percentage of the contraction in response to U46619, $n=8$, 6, 6 vessels. Tissue staining data are representative of 3 independent experiments (**D** and **H**). Values are mean $\pm$ SEM. Student t test (**A**, **B**, **E** through **G**, and **I**). Cumulative concentration-response curves were analyzed by comparing area under the curve with Student t test (**J**). HPLC-MS-MS indicates high-performance liquid chromatography-tandem mass spectrometry; PGE_2 , prostaglandin E_2 ; and $6\text{k-PGF}_{1\alpha}$, 6-keto-prostaglandin $\text{F}_{1\alpha}$.

ARTICLE INFORMATION

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Affiliations

State Key Laboratory of Cardiovascular Disease (H.C., Q.W., J.Y., H.R., C.X., P.X., X.Y., S.H., M.W.), Department of Pathology (H.W.), Department of Cardiovascular Surgery (W.F., L.W.), and NHC Key Laboratory of Clinical Research for Cardiovascular Medications, National Clinical Research Center of Cardiovascular Diseases (M.W.), Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing. Translational Cardiology, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden (M.B.). Department of Pharmacology, and Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia (R.E.W.). Shanghai Key Laboratory of Medical Epigenetics, International Co-Laboratory of Medical Epigenetics and Metabolism, Ministry of Science and Technology, Institutes of Biomedical Sciences, Fudan University, China (F.L.). Saha Cardiovascular Research Center and Department of Physiology, University of Kentucky, Lexington (H.S.L., A.D.). Department of Systems Pharmacology and Translational Therapeutics, Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia (G.A.F.). State Key Laboratory of Common Mechanism Research for Major Diseases, Department of Biochemistry and Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing (D.-P.L.). Department of Biomedical Sciences, The City University of Hong Kong, China (Y.H.). Department of Neurology, SUNY Downstate Medical Center, Brooklyn, NY (W.J.).

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Author Contributions

H.C., Q.W., J.Y., H.R., C.X., P.X. and X.Y. performed experiments and analyzed the data. S.H., H.W., W.F. and L.W. contributed essential materials. H.C., Q.W., J.Y., H.R., R.E.W., F.L., H.S.L., A.D., G.A.F., D.-P.L., Y.H., W.J. and M.M. contributed to the in-depth discussion of the results. H.C. and M.W. wrote the manuscript, and all authors revised the manuscript and approved the submission. M.W. conceived and supervised the project.

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Disclosures

G.A. FitzGerald is a Senior Advisor to Calico Laboratories. The other authors report no conflicts.

Supplemental Material

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Figures S1–S20
Videos S1–S4
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