

Chronic Angiotensin II Infusion Promotes Atherogenesis in Low Density Lipoprotein Receptor $-/-$ Mice

ALAN DAUGHERTY^a AND LISA CASSIS^{b,c}

^aDivision of Cardiovascular Medicine, Department of Medicine, and ^bDivision of Pharmaceutical Science, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536, USA

ABSTRACT: The purpose of this study was to determine the effect of chronic angiotensin II (AngII) infusion on the severity of the atherogenic process in low density lipoprotein (LDL) receptor $-/-$ mice with established lesions. LDL receptor $-/-$ mice receiving a diet enriched in cholesterol, saturated fat, and cholate, were infused with saline or AngII (500 ng/kg/min) for 28 days. Systolic blood pressure increased in LDL receptor $-/-$ mice following 7 days of AngII infusion, followed by a decline to baseline levels at 28 days, despite continued AngII infusion. Serum cholesterol was not influenced by AngII infusion in LDL receptor $-/-$ mice; however, serum triglyceride concentrations were reduced significantly in LDL receptor $-/-$ mice receiving AngII. The percent of intimal surface area covered by lesion was not increased in LDL receptor $-/-$ mice receiving AngII; however, the content of cholesterol (esterified and unesterified) in lesions of the arch, thoracic, and abdominal aorta was significantly increased in those mice infused with AngII. Of note, in 20% of the LDL receptor $-/-$ mice receiving AngII, large aneurysms were found in the abdominal aorta. Aneurysms appeared as breaks in the media and surrounding tissue of the vessel wall, encompassing amorphous and acellular masses with patches of thrombotic material. These results demonstrate that chronic infusion of AngII promotes the atherogenic processes in LDL receptor $-/-$ mice, manifest as increases in lesion cholesterol content. Effects of AngII to promote atherogenesis were apparent at doses which did not markedly elevate systolic pressure. Importantly, infusion of AngII in LDL receptor $-/-$ mice resulted in the development of aortic aneurysms.

Available evidence suggests that inhibition of angiotensin II (AngII), either by inhibitors of angiotensin converting enzyme (ACE) or AT1 receptor antagonists, exerts a beneficial effect on the atherogenic process. Direct proof that ACE inhibitors can retard the progression of atherosclerosis in humans is not available; however, published studies on the use of ACE inhibitors in patients with heart failure and after a myocardial infarction have demonstrated reductions in morbidity and mortality, potentially related to antiatherogenic effects.¹

A variety of studies have demonstrated reductions in the progression of atherosclerotic lesions in aortic tissue of animal models by inhibitors of ACE or AngII receptor blockers. Specifically, administration of ACE inhibitors resulted in reductions in the severity of atherosclerosis in monkeys,² pigs,³ rabbits,⁴ hamsters,⁵ and mice.^{6,7} Potential mechanisms contributing to the antiatherogenic effects of ACE inhibitors

^cAddress correspondence to Lisa A. Cassis, Ph.D., Room 434, College of Pharmacy, Rose Street, University of Kentucky, Lexington, KY 40536-0082. Tel.: (606) 257-4749; fax: (606) 257-7564.

include reductions in blood pressure, antiproliferative and antimigratory effects on vascular smooth muscle cells, neutrophils, and monocytes, restoration of endothelial function, stabilization of fatty plaque by prevention of vasoconstriction, and antiplatelet effects.⁸ Results from recent studies demonstrate that AngII influences the oxidative state of LDL, increasing LDL oxidation both *in vitro* (isolated macrophages) and *in vivo*.⁹ Moreover, AngII was shown to interact with AT1 receptors present on mouse peritoneal macrophages to increase 12/15-lipoxygenase mRNA expression, protein content, and enzymatic activity.¹⁰ Other mechanisms potentially contributing to AngII acceleration of the atherogenic process include AngII-induced elevations in mRNA abundance of monocyte chemoattractant protein (MCP-1), a chemokine implicated in recruitment of inflammatory cells to the atherosclerotic lesion.¹¹ Collectively, the available evidence supports a role for AngII in the progression of atherosclerosis. However, the majority of previous experimental studies have used indirect (effect of ACE inhibitors) or *in vitro* (AngII effects on cultured cells) approaches to study interactions between the renin-angiotensin system and atherosclerosis. Few studies have directly addressed the effect of elevations in systemic AngII concentrations on the atherogenic process in a model of atherosclerosis.

The purpose of this study was to determine the effect of chronic AngII infusion on the severity of the atherogenic process in LDL receptor *-/-* mice that had established lesions following diet-induced hyperlipidemia. The LDL receptor *-/-* mouse model of atherosclerosis was chosen because the lesion morphology is largely restricted to macrophage foam cells, a cell type previously suggested as a target site for AngII in the atherogenic process.^{12,13} We hypothesized that chronic exposure to elevated systemic concentrations of AngII will increase the severity of the atherogenic process in LDL receptor *-/-* mice.

METHODS

Mouse breeding and diet. LDL receptor *-/-* mice were obtained from Jackson Laboratories (Maine). The mice were originally generated as C57BL/6JXC129 hybrids, and mice used in this study were backcrossed 10 generations into a C57BL/6J background. Mice were housed in specific pathogen-free rooms and fed a normal mouse laboratory diet (Ralston Purina) until they were 2 months of age, after which they were fed a diet containing 1.25% cholesterol, 0.5% cholic acid, and 15% fat (Harlan Teklad, catalogue No. 88051) for three months. All procedures were approved by the University of Kentucky Animal Studies Committee.

Chronic AngII infusion. For studies in C57BL/6J mice, osmotic minipumps (Model 2001, 1.0 μ l/h, 7-day delivery, Alza, CA) containing either saline, or 50, 150 or 300 ng/kg/min of AngII (Sigma Chemical Co., St. Louis, MO) ($n = 4$ /dose) were implanted under metofane anesthesia. For studies in LDL receptor *-/-* mice, at 5 months of age (three months on the diet), LDL receptor *-/-* mice were anesthetized with metofane, and osmotic minipumps (Model 2004, 0.25 μ l/h, 28-day delivery) containing either saline ($n = 4$) or AngII (500 ng/kg/min; $n = 5$) were implanted.

Systolic blood pressure measurement. Systolic blood pressure was measured on metofane-anesthetized mice using an inflatable tail cuff which was connected in-line with a pressure transducer and a recording polygraph. Systolic pressure was mea-

sured at baseline (day 0, before minipump implantation), and on a weekly basis following the start of AngII infusion.

Serum lipid characterization. Serum total cholesterol and triglyceride concentrations were determined with enzymatic assay kits (Wako Chemical Company, Richmond, VA).

Quantification of atherosclerotic lesions. Aortic tissue was removed from the ascending aorta to the ileal bifurcation and placed in freshly prepared 4% paraformaldehyde in PBS overnight at room temperature. After tissue fixation, adventitial tissue was carefully removed. The intimal surface was exposed by a longitudinal cut through the inner curvature of the aortic arch that extended down the whole length of the aortic tree. To permit the arch region to be laid out flat, the greater curvature was cut down to the level of the left subclavian artery. The tissue was laid out on a black background and an image of the aorta was recorded.

To quantify the extent of intimal surface covered by grossly discernible lesions, image analysis was performed with Image-Pro (Media Cybernetics, Silver Springs, MD). Extent of atherosclerotic lesions was quantified in the arch, thorax, and abdominal regions as described previously.¹⁴ The regions were defined as follows: (a) arch, from the ascending arch to 4-mm distal to the left subclavian artery; (b) thorax, from the arch to the last intercostal artery branch; and (c) from the thorax to the branch of the ileal bifurcation. The percent intimal area covered by lesions was quantified by two observers.

Unesterified and esterified cholesterol content of the aortic regions described above was determined by enzymatic assay of lipid extracts. Tissue content of sterols ($\mu\text{g}/\text{mm}^2$) was normalized to the intimal surface area as determined by image analysis.

Statistical analysis. The null hypothesis for the variables measured in the two groups (LDL receptor $-/-$ and $+/-$ AngII) was initially tested by Student's *t* tests. If the data did not fit the constraints of this parametric test, data were analyzed with the Wilcoxon rank-sum test. All statistical analyses were performed by use of SigmaStat (Jandel Scientific, San Rafael, CA). Data are presented as means \pm SEM.

RESULTS

Chronic AngII infusion increases systolic pressure in C57BL/6J mice. To determine the dose-response relationship for the effect of chronic AngII infusion on systolic pressure, groups of C57BL/6J mice were infused with AngII (50, 150, or 300 ng/kg/min) or saline for seven days. In control mice receiving saline, systolic pressure did not vary over time (FIG. 1). Following seven days of AngII infusion, systolic pressure increased in a dose-dependent manner (FIG. 1). At the highest dose of AngII examined (300 ng/kg/min), systolic pressure increased by 33 mmHg in magnitude (55% increase over controls) at 7 days of infusion. Based on these results, an AngII infusion dose of 500 ng/kg/min, slightly higher than that used in preliminary studies, was chosen for studies in LDL receptor $-/-$ mice.

Chronic AngII infusion modestly elevates systolic pressure in LDL receptor $-/-$ mice. The infusion of AngII (500 ng/kg/min) for 28 days produced a modest hypertensive effect in LDL receptor $-/-$ mice (FIG. 2). The degree of hypertension was

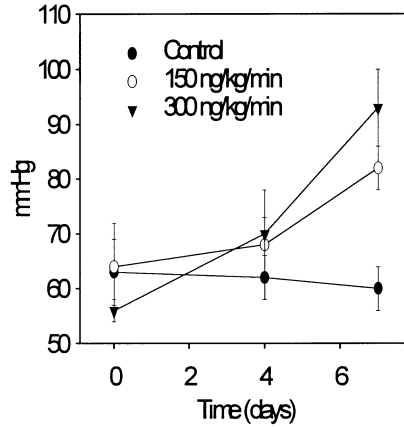


FIGURE 1. Chronic AngII infusion increases systolic pressure in C57BL/6J mice. Three groups of C57BL/6J mice were implanted with osmotic minipumps containing either saline, AngII 150 ng/kg/min, or AngII 300 ng/kg/min for 7-day delivery. Systolic pressure remained constant in saline-infused mice over the 7-day protocol. Infusion of AngII at both doses significantly ($p < 0.0005$) increased systolic pressure by approximately 33 mmHg in magnitude on day 7. Data are mean \pm SEM from $n = 4$ mice/group.

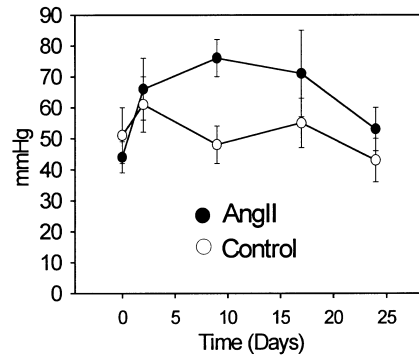


FIGURE 2. Chronic AngII infusion modestly elevates systolic pressure in LDL receptor $-/-$ mice. At 5 months of age, and following two months of high cholesterol diet, LDL receptor $-/-$ mice were implanted with osmotic minipumps containing either saline or AngII (500 ng/kg/min) for 28-day delivery. Chronic infusion of AngII resulted in a significant ($p < 0.05$) increase in systolic pressure at 10 days of infusion, which returned to levels not significantly different from control or baseline (day 0) by 18 days of infusion. Data are mean \pm SEM from $n = 4$ saline, $n = 5$ LDL receptor $-/-$ mice.

slightly less than anticipated based on results in C57BL/6J mice receiving AngII (FIG. 1). Moreover, blood pressure elevation in response to AngII was not maintained over the 28-day infusion protocol, falling to baseline pressure (day 0) by 4 weeks of AngII infusion.

Chronic AngII infusion increases the aortic content of cholesterol in atherogenic lesions. Following chronic AngII infusion, serum cholesterol concentrations were not altered in LDL receptor $-/-$ mice (FIG. 3). However, serum triglyceride concentrations were significantly decreased in LDL receptor $-/-$ mice receiving AngII (FIG. 4). The thickness of grossly discernible lesions was noticeably greater in AngII-infused mice; however, quantification of the percent of surface aortic intima covered by lesions was not significantly different in LDL receptor $-/-$ mice receiving AngII (FIG. 5). In support of the observation of an apparent increase in the thickness of aortic lesions by visual inspection, the content of cholesterol was significantly increased in atherogenic lesions of the arch, thoracic and abdominal aorta from AngII-infused mice (FIG. 6). Elevations in aortic cholesterol in lesions from AngII-infused mice were the result of increases in both esterified and unesterified cholesterol (FIG. 6).

Chronic AngII infusion results in abdominal aortic aneurysms in LDL receptor $-/-$ mice. In two of the five mice receiving AngII, readily discernible, large aneurysms were noted in the abdominal aorta (FIG. 7). The illustrated aorta from an AngII-infused LDL receptor $-/-$ mouse exhibited a break in the media and surrounding tissue of the vessel wall, encompassing an amorphous and acellular mass with patches of thrombotic material.

DISCUSSION

The major findings of this study are that chronic AngII infusion increased the cholesterol content of atherogenic lesions, and resulted in the appearance of large

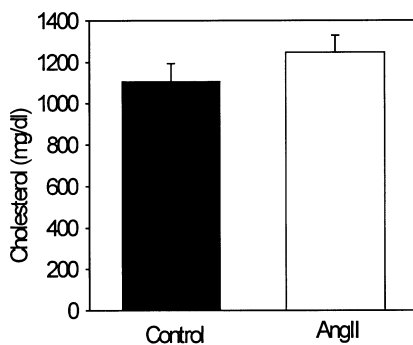


FIGURE 3. Chronic AngII infusion does not alter serum cholesterol concentrations. Serum total cholesterol concentrations were determined with an enzymatic kit. Following 28 days of AngII (500 ng/kg/min) infusion in LDL receptor $-/-$ mice, serum cholesterol concentrations were not significantly altered. Data are mean \pm SEM from $n = 4$ saline, $n = 5$ LDL receptor $-/-$ mice.

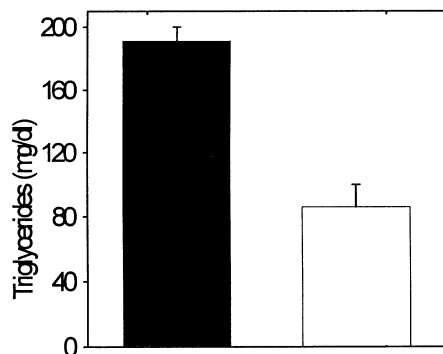


FIGURE 4. Chronic AngII infusion decreases serum triglyceride concentrations. Serum triglyceride concentrations were determined with an enzymatic kit. Following 28 days of AngII (500 ng/kg/min) infusion in LDL receptor $-/-$ mice, serum triglyceride concentrations were significantly ($p < 0.05$) decreased in mice receiving AngII. Data are mean \pm SEM from $n = 4$ saline, $n = 5$ LDL receptor $-/-$ mice.

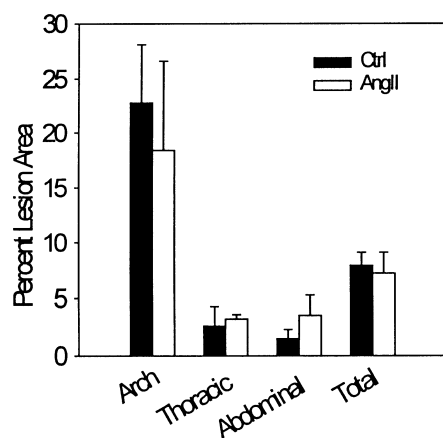


FIGURE 5. Percent of surface aortic intima covered by atherogenic lesions. Quantification of the extent of intimal surface area covered by grossly discernible lesions was as described in METHODS. Following 28 days of AngII (500 ng/kg/min) infusion in LDL receptor $-/-$ mice, the percent of surface intima covered by atherogenic lesions in the arch, thoracic, and abdominal aorta was not significantly different between saline-infused and AngII-infused mice. Data are mean \pm SEM from $n = 4$ saline, $n = 5$ LDL receptor $-/-$ mice.

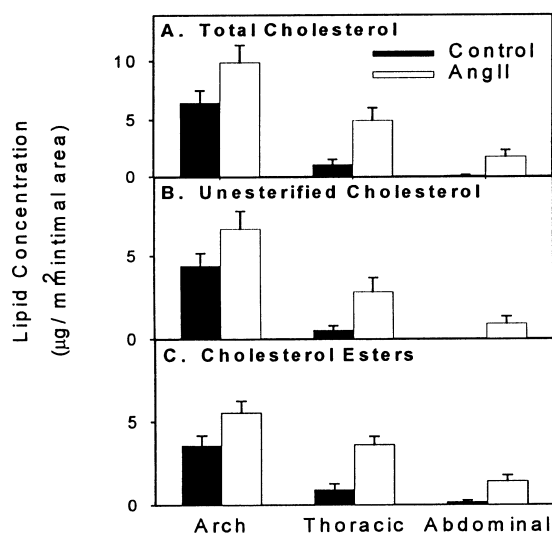


FIGURE 6. Chronic AngII infusion increases the aortic content of cholesterol in atherosclerotic lesions from LDL receptor $-/-$ mice. Total cholesterol, unesterified cholesterol, and cholesterol esters in atherosclerotic lesions were determined by enzymatic assay of lipid extracts. Following 28 days of AngII infusion, cholesterol (unesterified and esterified) content in atherosclerotic lesions from the arch, thoracic, and abdominal aorta was significantly ($p < 0.05$) increased in mice receiving AngII. Data are mean \pm SEM from $n = 4$ saline, $n = 5$ LDL receptor $-/-$ mice.

aneurysms in the abdominal aorta of LDL receptor $-/-$ mice, in the absence of marked elevations in systolic blood pressure. To our knowledge, this is the first direct *in vivo* evidence for promotion of the atherosclerotic process in response to a chronic infusion of AngII.

Several laboratories have examined the effect of Goldblatt hypertension, with associated alterations in the systemic renin-angiotensin system, on the incidence and severity of atherosclerotic lesions. Marked increases (77%) in the aortic surface area covered by atherosclerotic disease, concomitant with elevations in systolic pressure, were demonstrated using the one-kidney, one-clip Goldblatt model of hypertension in the Watanabe rabbit.⁴ This model of hypertension is associated with initial increases in plasma renin activity and presumably systemic AngII concentrations, followed by a gradual return to normal levels over time. Further studies in the same model demonstrated an increase in atherosclerotic involvement specifically at the coronary arteries following three to six months of hypertension.¹⁵ Hypertension was associated with extensive and complicated atheromas, as demonstrated using the two-kidney, one-clip Goldblatt model of high renin hypertension in New Zealand rabbits fed a high cholesterol diet.¹⁶ Recent studies in transgenic mice carrying both the human renin and angiotensinogen genes demonstrated that administration of an atherosclerotic diet resulted in accelerated damage of cellular structure in the aortic root, culminating in a fourfold larger surface area of atherosclerotic lesions.¹⁷ Collectively, these

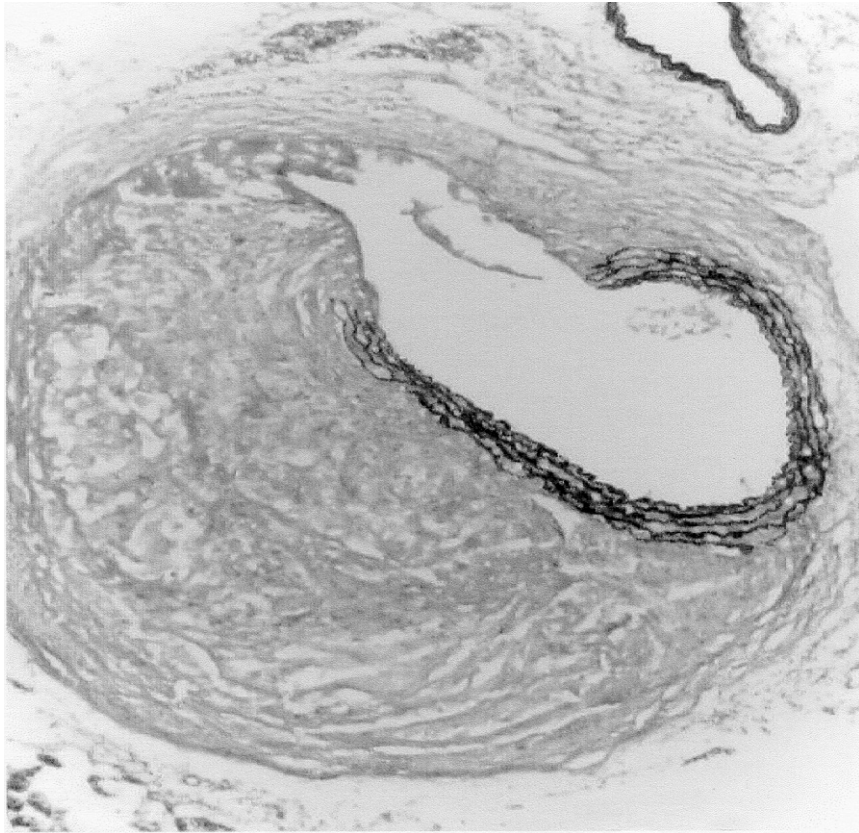


FIGURE 7. Chronic AngII infusion results in abdominal aortic aneurysms in LDL receptor $-/-$ mice. A cross section, stained for elastin (black), of a representative abdominal aortic aneurysm from an LDL receptor $-/-$ mouse receiving AngII. The medial layer of the aortic wall is broken, with marked hyperplasia, acellular masses, and patches of thrombotic material. Aneurysms were not detected in LDL receptor $-/-$ mice receiving saline.

results suggest that hypertension associated with elevated concentrations of systemic AngII increase the severity of atherogenic lesions. Confirming and extending results from previous studies, our results directly demonstrate that elevations in systemic AngII concentrations promote the atherogenic process.

Interestingly, increases in aortic cholesterol content of intimal lesions in LDL receptor $-/-$ mice were observed following infusion of doses of AngII that did not markedly increase systolic pressure. Our preliminary results examining chronic AngII infusion in C57BL/6J mice demonstrated dose-dependent elevations in systolic pressure following seven days of AngII infusion. Of importance, infusion of AngII at a dose of 300 ng/kg/min in C57BL/6J mice resulted in a 55% increase in systolic pressure over baseline. With a dose of 500 ng/kg/min of AngII infusion in

LDL receptor $-/-$ mice, systolic pressure was increased by 55% at 7 days of AngII infusion. These results suggest that the ability of AngII to increase systolic pressure in cholesterol-fed LDL receptor $-/-$ mice was blunted compared to C57BL/6J mice. Moreover, the effect of AngII to elevate systolic pressure in LDL receptor $-/-$ mice was not maintained over the 28-day infusion protocol. Future studies will determine mechanisms that give rise to blunted hemodynamic responses to AngII in the LDL receptor $-/-$ mouse model. Of note, promotion of the atherogenic process in LDL receptor $-/-$ mice in the present study was observed in the absence of marked or prolonged increases in systolic pressure following AngII infusion.

Our results demonstrate that AngII infusion increased the cholesterol content of atherogenic lesions in LDL receptor $-/-$ mice. Mechanisms for AngII-mediated increases in cholesterol content of aortic lesions were not identified in the present study. Previous results demonstrated that chronic infusion of AngII for seven days into rats increased mRNA abundance for MCP-1, a chemokine implicated in the recruitment of monocytes/macrophages to the atherosclerotic lesion.^{18,19} Future studies will determine whether MCP-1 serves as a mediator contributing to AngII promotion of the atherogenic process.

Of particular note in the present study was the appearance of large aneurysms in the abdominal aorta of LDL receptor $-/-$ mice receiving AngII. Aneurysms were not present in C57BL/6J mice receiving AngII, and appeared to be related to the LDL receptor $-/-$ phenotype. Aneurysms appeared as breaks in the medial arterial layer, suggesting weakening of the vascular wall, and were not associated with atherogenic lesions. Previous studies have noted mild dilations that may presage fully formed aneurysms in apoE $-/-$ mice.²⁰ This dilation is inhibited by the absence of urokinase plasminogen activator due to inhibition of metalloelastase.²¹ Future studies will address mechanisms that give rise to AngII-induced aneurysms and their relationship to the atherogenic process.

In conclusion, results from this study demonstrate that chronic infusion of AngII promotes the atherogenic process in LDL receptor $-/-$ mice, in the absence of marked elevations in systolic pressure. Promotion of atherogenesis in LDL receptor $-/-$ mice receiving AngII were manifest as increases in the cholesterol content of atherogenic lesions. Interestingly, infusion of AngII in LDL receptor $-/-$ mice resulted in the development of large aneurysms in the abdominal aortic wall. Future studies will determine mechanisms that give rise to AngII-mediated increases in atherogenesis and the development of aortic aneurysms.

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REFERENCES

1. PFEFFER, M. A., E. BRAUNWALD, L. A. MOYE, F. BASTA, E. J. BROWN, T. E. CUDDY, B. R. DAVIS, E. M. GELTMAN, S. GOLDMAN, G. C. FLAKER, M. KLEIN, G. A. LAMAS, M. PACKER,

- J. ROULEAU, J. L. ROULEAU, J. RUTHERFORD, J. H. WERTHEIMER & C. M. HAWKINS. 1992. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. *N. Engl. J. Med.* **327**: 669–677.
2. ABERG, G. & P. FERRER. 1990. Effects of captopril on atherosclerosis in cynomolgus monkeys. *J. Cardiovasc. Pharmacol.* **15**: S65–S72.
 3. CHARPIOT, P., P. H. ROLLAND, A. FRIGGI, P. PIQUET, E. SCALBERT, H. BODARD, A. BARLATIER, V. LATRILLE, P. TRANIER, C. MERCIER, R. LUCCIONI, R. CALAF & D. GARCON. 1993. ACE Inhibition with perindopril and atherogenesis-induced structural and functional changes in minipig arteries. *Arteriosclerosis and Thrombosis* **13**: 1125–1138.
 4. CHOBANIAN, A. V., C. C. HAUDENSCHILD, C. NICKERSON & R. DRAGO. 1990. Antiatherogenic effect of captopril in the Watanabe heritable hyperlipidemic rabbit. *Hypertension* **15**: 327–331.
 5. KOWALA, M. C., R. I. GROVE & G. ABERG. 1994. Inhibitors of angiotensin converting enzyme decrease early atherosclerosis in hyperlipidemic hamsters. Fosinopril reduces plasma cholesterol and captopril inhibits macrophage-foam cell accumulation independently of blood pressure and plasma lipids. *Atherosclerosis* **108**: 61–72.
 6. KEIDAR, S., J. ATTIAS, J. SMITH, J. L. BRESLOW & T. HAYEK. 1997. The angiotensin-II receptor antagonist, losartan, inhibits LDL lipid peroxidation and atherosclerosis in apolipoprotein E-deficient mice. *Biochem. Biophys. Res. Commun.* **236**: 622–625.
 7. HAYEK, T., J. ATTIAS, J. SMITH, J. L. BRESLOW & S. KEIDAR. 1998. Antiatherosclerotic and antioxidative effects of captopril in apolipoprotein E-deficient mice. *J. Cardiovasc. Pharmacol.* **31**: 540–544.
 8. CHENG, J. W., & M. N. NGO. 1997. Current perspective on the use of angiotensin-converting enzyme inhibitors in the management of coronary (atherosclerotic) artery disease. *Ann. Pharmacother.* **31**: 1499–1506.
 9. KEIDAR, S. 1998. Angiotensin, LDL peroxidation and atherosclerosis. *Life Sci.* **63**: 1–11.
 10. SCHEIDEGGER, K. J., S. BUTLER & J. L. WITZTUM. 1997. Angiotensin II increases macrophage-mediated modification of low density lipoprotein via a lipoxygenase-dependent pathway. *J. Biol. Chem.* **272**: 21609–21615.
 11. CHEN, X. L., P. E. TUMMALA, M. T. OLBRYCH, R. W. ALEXANDER & R. M. MEDFORD. 1998. Angiotensin II induces monocyte chemoattractant protein-1 gene expression in rat vascular smooth muscle cells. *Circ. Res.* **83**: 952–959.
 12. ISHIBASHI, S., J. L. GOLDSTEIN, M. S. BROWN, J. HERZ & D. K. BURNS. 1994. Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. *J. Clin. Invest.* **93**: 1885–1893.
 13. ROSELAAR, S. E., P. X. KAKKANATHU & A. DAUGHERTY. 1996. Lymphocyte populations in atherosclerotic lesions of ApoE $-/-$ and LDL receptor $-/-$ mice—Decreasing density with disease progression. *Arterioscler. Thromb. Vasc. Biol.* **16**: 1013–1018.
 14. DAUGHERTY, A., E. PURE, D. DELFEL-BUTTEIGER, S. CHEN, J. LEFEROVICH, S. E. ROSELAAR & D. J. RADER. 1997. The effects of total lymphocyte deficiency on the extent of atherosclerosis in apolipoprotein E $-/-$ mice. *J. Clin. Invest.* **100**: 1575–1580.
 15. NICKERSON, C. J., C. C. HAUDENSCHILD & A. V. CHOBANIAN. 1992. Effects of hypertension and hyperlipidemia on the myocardium and coronary vasculature of the WHHL rabbit. *Exp. Mol. Pathol.* **56**: 173–185.
 16. OVERTURE, M. L., C. ASCHENBRENER, R. E. DRUILHET & W. M. KIRKENDALL. 1981. Renin as a risk factor for atherogenesis. Effects of hypercholesterolemia and two-kidney—one-clip hypertension in the rabbit. *Atherosclerosis* **38**: 97–119.
 17. SUGIYAMA, F., S. HARAOKA, T. WATANABE, N. SHIOTA, K. TANIGUCHI, Y. UENO, K. TANIMOTO, K. MURAKAMI, A. FUKAMIZU & K. YAGAMI. 1997. Acceleration of atherosclerotic lesions in transgenic mice with hypertension by the activated renin-angiotensin system. *Lab. Invest.* **76**: 835–842.

18. BORING, L., J. GOSLING, M. CLEARY & I. F. CHARO. 1998. Decreased lesion formation in CCR2(-/-) mice reveals a role for chemokines in the inhibition of atherosclerosis. *Nature* **394**: 894–897.
19. GU, L., Y. OKADA, S. K. CLINTON, C. GERARD, G. K. SUKHOVA, P. LIBBY & B. J. ROLLINS. 1998. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol. Cell* **2**: 275–281.
20. TANGIRALA, R. K., E. M. RUBIN & W. PALINSKI. 1995. Quantitation of atherosclerosis in murine models: correlation between lesions in the aortic origin and in the entire aorta, and differences in the extent of lesions between sexes in LDL receptor-deficient and apolipoprotein E-deficient mice. *J. Lipid Res.* **36**: 2320–2328.
21. CARMELIET, P., L. MOONS, R. LJNEN, M. BAES, V. LEMAITRE, P. TIPPING, A. DREW, Y. ECKHOUT, S. SHAPIRO, F. LUPU & D. COLLEN. 1997. Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. *Nature Genet.* **17**: 439–444.