Published in final edited form as:

Genet Med. 2012 October ; 9(5): 319–328. doi:10.1016/j.genm.2012.08.003.

Endogenous Androgen Deficiency Enhances Diet-Induced Hypercholesterolemia and Atherosclerosis in Low-Density Lipoprotein Receptor-Deficient Mice

Nicholas W. Hatch, PhD¹, Sarah J. Srodulski, BS², Huei-Wei Chan, PhD², Xuan Zhang, PhD¹, Lisa R. Tannock, MD^{1,3,4}, and Victoria L. King, PhD^{1,2,4}

¹Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, Kentucky

²Division of Cardiovascular Medicine, University of Kentucky, Lexington, Kentucky

³Division of Endocrinology, University of Kentucky, Lexington, Kentucky

⁴Barnstable Brown Diabetes and Obesity Center, University of Kentucky, Lexington, Kentucky

Abstract

Background—Despite numerous clinical and animal studies, the role of sex steroid hormones on lipoprotein metabolism and atherosclerosis remain controversial.

Objective—We sought to determine the effects of endogenous estrogen and testosterone on lipoprotein levels and atherosclerosis using mice fed a low-fat diet with no added cholesterol.

Methods—Male and female low-density lipoprotein receptor-deficient mice were fed an open stock low-fat diet (10% of kcals from fat) for 2, 4, or 17 weeks. Ovariectomy, orchidectomy, or sham surgeries were performed to evaluate the effects of the presence or absence of endogenous hormones on lipid levels, lipoprotein distribution, and atherosclerosis development.

Results—Female mice fed the study diet for 17 weeks had a marked increase in levels of total cholesterol, triglycerides, apolipoprotein-B containing lipoproteins, and atherosclerosis compared with male mice. Surprisingly, ovariectomy in female mice had no effect on any of these parameters. In contrast, castration of male mice markedly increased total cholesterol concentrations, triglycerides, apolipoprotein B-containing lipoproteins, and atherosclerotic lesion formation compared with male and female mice.

Conclusions—These data suggest that endogenous androgens protect against diet-induced increases in cholesterol concentrations, formation of proatherogenic lipoproteins, and atherosclerotic lesions formation. Conversely orchidectomy, which decreases androgen concentrations, promotes increases in cholesterol concentrations, proatherogenic lipoprotein formation, and atherosclerotic lesion formation in lowdensity lipoprotein receptor-deficient mice in response to a low-fat diet.

© 2012 Excerpta Medica Inc. All rights reserved.

Address correspondence to: Victoria L. King, PhD, Department of Internal Medicine, Division of Cardiovascular Medicine, University of Kentucky, 900 S Limestone St, Rm 562, Lexington, KY 40536-0200. vicky.king@uky.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article.

Keywords

androgen; atherosclerosis; cholesterol; estrogen

INTRODUCTION

Cardiovascular disease remains the leading cause of death in both men and women in the United States. However, the effect of sex and sex hormones on atherosclerosis remains controversial. In premenopausal women endogenous estrogen is thought to be atheroprotective in part due to its beneficial effects on lipid levels, as well as possible direct effects on the vascular wall (this has been reviewed elsewhere¹⁻³). Clinical trials have suggested that the loss of estrogen in postmenopausal women leads to alterations in lipoprotein profiles and increased cardiovascular disease events. Thus, for many years estrogen was prescribed to postmenopausal women with the belief that it would provide cardioprotection. However, long-term clinical studies investigating hormone replacement therapy in postmenopausal women have demonstrated that hormone replacement therapy did not provide cardiovascular protection and actually increased cardiovascular events in some women, especially during the first year of use.⁴⁻⁶ Subsequent analyses have suggested that cardiovascular risk with hormone replacement therapy is related to timing of use after onset of menopause⁷ or baseline lipid levels. In the Women's Health Initiative study women with an LDL:HDL ratio <2.5 had no increase in cardiovascular disease with hormone replacement, but women with an LDL:HDL ratio >2.5 did have increased risk.⁸

In contrast, testosterone is considered to have adverse effects on cardiovascular risk in part due to its effects to reduce HDL cholesterol.^{9,10} Although this is the current dogma, low levels of testosterone have been associated with proatherogenic lipoprotein profiles and increased incidence of stroke and ischemia.^{11,12} Recent data suggests that as testosterone levels decrease in men there is an increase in risk for cardiovascular disease as well as for metabolic perturbations that are associated with an increased risk for cardiovascular disease.¹³⁻¹⁵ Testosterone levels negatively associate with carotid intima-media thickening and atherosclerosis.^{9,10} Moreover, animal studies suggest that testosterone suppresses foam cell formation and reduces atherosclerosis lesions, supporting a cardioprotective function of testosterone.^{16,17}

Despite numerous studies investigating sex effects on atherosclerosis in both human beings and animal models, our understanding of sex differences in atherosclerosis is incomplete. Many studies have used administration of exogenous sex steroids in an effort to understand sex steroid and sex effects,¹⁶⁻¹⁹ but these approaches do not directly correlate with loss of endogenous hormones. For example, administration of testosterone in animal models has been shown to reduce the development of atherosclerosis,²⁰⁻²² but these effects may be due to its conversion to estradiol.²¹ Similarly, administration of exogenous estrogen can be confounded by potential effects on thrombogenesis. Therefore, we investigated the effect of endogenous estrogen and androgen on cholesterol concentrations, lipoprotein distribution, and atherosclerotic lesion formation. We used a murine model with a humanized lipoprotein profile and fed them a low-fat diet. Herein we demonstrate that androgens protect against the development of atherosclerotic lesion formation in low-density lipoprotein receptor-deficient (LDLR^{-/-}) mice, via maintaining lower levels of apolipoprotein B (apoB)-containing lipoproteins. In response to orchidectomy, which results in a marked reduction in endogenous testosterone, apoB-containing lipoproteins increased with a concomitant increase in atherosclerosis. These data suggest that reductions in endogenous androgen concentrations may promote a proatherogenic lipoprotein profile and atherosclerotic lesion formation.

MATERIALS AND METHODS

Animal Model

Male and female LDLR^{-/-} mice backcrossed 10 times onto a C57BL/6 background were obtained from the Jackson Laboratories (Bar Harbor, Maine). Mice were housed in specific pathogen-free rooms and fed a normal laboratory diet (2918, Harlan Teklad, Indianapolis, Indiana) before commencement of the studies. All studies were approved by the University of Kentucky Institutional Animal Care and Use Committee. Male and female LDLR^{-/-} mice aged 8 weeks were placed on diet containing 10% of kcals from fat (D12450B; Research Diets, New Brunswick, New Jersey) for 2, 4, or 17 weeks. In some experiments mice aged 8 weeks were subjected to ovariectomy, orchidectomy, or sham surgeries as described previously.²³ Following a 4-week recovery period mice received the study diet for 17 weeks. Age-matched control groups of the opposite sex were used in all ovariectomy and orchidectomy studies to control for variability in cholesterol concentrations and atherosclerotic lesion formation, as well as to determine if the treatment modality altered these parameters to the level of that observed in the opposite sex.

Biochemical Measurements

Testosterone concentrations were determined using Active Testosterone RIA DSL-4000 kit (Diagnostic Systems, Inc, Webster, Texas). Plasma cholesterol and triglyceride concentrations were measured by enzymatic colorimetric assays (Wako Chemical Co, Richmond, Virginia) as previously described.²⁴ Plasma aliquots from individual mice were separated by fast protein liquid chromatography as previously described²⁵ and cholesterol and triglyceride content of each fraction was quantified by enzymatic colorimetric assays (Wako Chemical Co) as previously described.²⁵

Atherosclerosis Quantification

Mouse aortas were removed and fixed in 10% formalin overnight. Aortas were cleaned, cut, pinned, and photographed for en face measurements of atherosclerosis. Images of aortas were captured by digital camera (Nikon DXM1200, Nikon, Melville, New York). The area of discernible lesions on the intimal surface of the aortic arch were measured by NIS Elements (Nikon) and normalized to aortic arch area as previously described.²⁵

Statistical Analysis

Data were analyzed by the Student *t* test or analysis of variance, as appropriate. Significant interactions identified by analysis of variance were analyzed using a Bonferroni post hoc test. Nonparametric data was analyzed by Mann-Whitney Rank sum test or significant interactions were analyzed by using a Holm-Sidak multiple comparisons, where appropriate. All data analyses were performed using SigmaStat software (IBM Corp, Armonk, New York). All data are represented as means (SEM). *P* < 0.05 was considered statistically significant.

RESULTS

Sex-Specific Effects of Feeding a Diet Moderately Enriched in Saturated Fat on Lipid Concentrations and Atherosclerotic Lesion Formation

To investigate the effect of sex on lipid concentrations we utilized a diet providing 10% of kcals as fat that contained purified ingredients that are highly refined and have little variability between lots. In comparison, nonpurified diets (such as standard rodent chow diets used in a majority of studies) contain plant-derived ingredients that may vary the nutritional content of the diet from lot to lot due to seasonal changes in plant-derived content.

Moreover, the plant-derived ingredients used in standard rodent chow diets and many “Western diets” contain phytoestrogens that are not present in the diet used in our studies. The source of fat in our study diet is lard and soybean oil, with 20% of the fat being saturated; thus, the final saturated fat in the diet is 4%. Moreover, this study diet contains no added cholesterol. Male and female LDLR^{-/-} mice aged 8 weeks were fed the study diet for 17 weeks. There were no differences between sexes in weight gain (Table I); however, as expected male mice weighed more than female mice. Although cholesterol concentrations and lipoprotein distribution were the same between sexes at study initiation (Figures 1A and 1B), cholesterol concentrations increased within 1 week of study diet consumption (Figure 1A) and remained significantly higher in female mice compared with male mice following feeding the diet for 17 weeks (Table II). Lipoprotein distribution was measured by fast protein liquid chromatography and VLDL and LDL concentrations were significantly increased in female mice compared with male mice within 1 week of study diet consumption (Figure 1C) and remained elevated for 17 weeks (Figure 1D). In concordance with the increase in apoB-containing lipoproteins, there was a marked increase in atherosclerotic lesion formation in female mice compared with male mice (Figure 1E).

Ovariectomy has no Effect on Plasma Lipid Levels and Atherosclerotic Lesion Formation

To determine if female sex hormones mediated the sex-specific effects on lipid levels and atherosclerotic lesion formation, female LDLR^{-/-} mice aged 8 weeks were either ovariectomized or had sham surgery. Four weeks after surgery, sham female, ovariectomized female, and male mice were fed the study diet for 17 weeks. At termination of the study, there was a 4-fold reduction in uterus weight in ovariectomized mice compared with sham female mice (Figure 2A), reflecting a marked reduction in estrogen following ovariectomy. As expected,^{26–28} ovariectomized female mice gained significantly more weight than female sham mice ($P < 0.005$); moreover, the body weight of the ovariectomized female mice was similar to that of the male mice (Table I). There was no effect of ovariectomy on plasma cholesterol concentrations, triglyceride concentrations (Table II), or lipoprotein distribution in female mice (Figures 2B and 2C). In accord with these findings, ovariectomy modestly attenuated atherosclerotic lesion formation compared with sham female mice (Figure 2D); however, both groups of female mice had significantly more atherosclerosis than male mice (Figure 2D).

Orchidectomy Increases Serum Cholesterol Level, Proatherogenic Lipoprotein Formation, and Atherosclerotic Lesion Formation

To determine if male sex hormones were protective against hypercholesterolemia, proatherogenic lipoprotein formation, and atherosclerosis, male mice aged 8 weeks were orchidectomized or had sham surgery. Four weeks after surgery, orchidectomized males, sham males, and female mice were fed the study diet for 17 weeks. At the termination of the study, orchidectomized mice had a 5-fold reduction in testosterone concentrations compared with sham male mice; moreover, plasma testosterone levels in orchidectomized mice were lower than those of female mice (Figure 3A). Orchidectomy attenuated weight gain compared with sham male mice ($P < 0.001$); moreover, the body weight gain of the orchidectomized male mice was similar to that of the female mice (Table I). In addition, orchidectomy increased plasma cholesterol and triglycerides (Table II) as well as increased cholesterol and triglyceride lipoprotein distribution into apoB-containing lipoproteins compared with either sham male or female mice (Figures 3B and 3C). This increase in plasma lipids after orchidectomy was seen as early as 2 weeks after diet initiation (data not shown). The increase in proatherogenic lipoprotein formation in the orchidectomized group resulted in a concomitant increase in atherosclerotic lesion formation compared with both sham male and female mice (Figure 3D). Moreover, these data demonstrate that sex

hormone regulation of cholesterol concentrations correlate with atherosclerotic lesion formation ($P < 0.0001$) (data not shown).

DISCUSSION

The role of the sex steroid hormones estrogen and testosterone in reducing or increasing the risk of cardiovascular disease is still under much debate. Many studies have suggested that estrogen is cardioprotective and the loss of estrogen during menopause increases the risk of cardiovascular disease. However, more recent studies have suggested that loss of testosterone may also promote cardiovascular risk. Atherosclerosis develops over a lifetime even in lean individuals who eat a healthy diet. Therefore, we sought to determine the effect of endogenous sex hormones on atherosclerosis in response to feeding a diet that provided 10% of kcals from fat that was moderately enriched in saturated fat (ie, a healthy diet similar to a healthy human diet).

Cholesterol and triglyceride concentrations as well as concentrations of the apoB-containing lipoproteins VLDL and LDL were significantly increased in female mice compared with male in response to the study diet. The increase in these apoB-containing lipoproteins was concomitant with a marked increase in atherosclerotic lesion formation in female mice. Ovariectomy had no effect of cholesterol concentrations, lipoprotein distribution, or atherosclerosis; in contrast, orchidectomy increased all of these parameters. These findings are in accord with clinical data suggesting that low testosterone levels in males correlates with an atherogenic lipid profile and increased risk of cardiovascular disease.^{9,10}

The diet we used is a low-fat diet that contains a negligible amount of cholesterol, with the source of the fat in this diet being lard and soybean oil. The diet also contains carbohydrate and protein. This diet is commonly used as the low-fat control diet for diet-induced obesity studies where the effects of feeding this diet are compared with feeding either a 45% of kcals from fat diet or 60% of kcals from fat diet.^{24,29–31} These diets contain the exact same constituents; however, the concentrations of the fat and carbohydrate constituents are altered to obtain the increase in percent kcals from fat in the diet. Moreover, this is a purified diet that excludes the confounding effect of variability from lot to lot. It is conceivable that male and female mice may respond to saturated fat ingestion differently. Although we cannot rule out this possibility without further investigation, we found no differences in total cholesterol and triglyceride concentrations or lipoprotein distribution between the sham and ovariectomized females, thereby suggesting that estrogen is not affecting absorption of the constituents in the diet.

Estrogen may exert direct effects on vascular walls (this is reviewed elsewhere³). Studies investigating the role of endogenous estrogen on atherosclerosis have provided contradictory results. Some studies have demonstrated that ovariectomy attenuated atherosclerotic lesion formation,³² other studies have demonstrated that ovariectomy increased atherosclerotic lesion formation,^{33–35} whereas other studies have demonstrated that ovariectomy had no effect of atherosclerotic lesion formation.^{23,36} Each of these various studies used different diets containing different concentrations of added cholesterol (0.15%–1.25% added cholesterol) and fat (9.0%–42% of kcals), which may have contributed to the different outcomes observed. In addition, the source of protein in the diet also has a significant influence on atherosclerotic lesion formation in ovariectomized mice.³⁷ However, 1 study demonstrated that ovariectomy attenuated lesion formation in apolipoprotein E-deficient mice fed a chow diet (14% of kcals from fat [0.02% cholesterol]) for 12 weeks. With the exception of that article,³³ most studies did not compare the effect of gonadectomy, and most of the studies were focused on the effect of estradiol replacement on atherosclerotic lesion formation. In contrast, we specifically examined the effect of reductions in

endogenous sex hormone concentrations in response to gonadectomy compared with intact females and males in mice fed a diet compositionally similar to a healthy human diet and demonstrated that reductions in female sex hormones in response to ovariectomy does not increase atherosclerotic lesion formation. The difference between our findings and studies demonstrating either no effect or increases or decreases in lesion formation in response to ovariectomy may be due to differences in diets, in background strain, murine models used, duration of diets, and lack of comparison to sham surgery in a majority of the studies.

In agreement with our findings, Nathan et al²¹ demonstrated that orchidectomy in LDLR^{-/-} mice resulted in a marked increase in atherosclerotic lesion formation. Those authors suggested that the increased lesion formation in ovariectomized mice was due to a loss of estradiol production in the male mice. However, aromatase inhibition only had minimal effect on lesion formation.¹⁹ Qui et al¹⁷ demonstrated that orchidectomy increased atherosclerotic lesion formation in rabbits and treatment with dihydrotestosterone, an androgen that cannot be aromatized, suppressed lesion formation in the orchidectomized rabbits, suggesting that the protective role of testosterone may not be due to aromatization of testosterone to estradiol. In our study, female mice had higher testosterone levels and less atherosclerosis than orchidectomized mice. Although we cannot discount that aromatization of testosterone to estradiol accounts for the difference in lesion formation between the female mice and orchidectomized male mice, our data also demonstrate that ovariectomy had no effect on atherosclerosis. Therefore, our data do not support a role for female sex hormones in preventing atherosclerotic lesion formation. In contrast, our data suggest that androgens are atheroprotective in LDLR^{-/-} mice.

Clinical data suggests that testosterone affects both cholesterol and lipoprotein concentrations. Animal studies investigating the role of testosterone on atherosclerotic lesion formation have demonstrated conflicting effects of testosterone on cholesterol concentrations and lipoprotein distribution. Early studies demonstrated that testosterone administration to castrated C57BL/6 mice decreased cholesterol and apoB-containing lipoprotein concentrations.³⁸ However, later studies by Nathan et al²¹ found that orchidectomy did not alter cholesterol and lipoprotein concentrations in LDLR^{-/-} mice fed a high-fat diet. More recent studies demonstrated an increase in cholesterol concentrations and apoB-containing lipoproteins in orchidectomized apolipoprotein E-deficient mice that are deficient in the androgen receptor.¹⁶ Moreover, treatment of the orchidectomized mice with testosterone reduced cholesterol and VLDL concentrations. Our findings of increased cholesterol concentrations and apoB-containing lipoproteins in LDLR^{-/-} mice are in accord with these findings. With the exception of our studies, other studies have all utilized highfat diets enriched in cholesterol, whereas we utilized a healthy diet moderately enriched in saturated fat with no added cholesterol. This diet resulted in an increased accumulation of cholesterol and apoB-containing lipoproteins in female and ovariectomized female LDLR^{-/-} mice compared with male LDLR^{-/-} mice. These results suggest that feeding a high-fat diet enriched in cholesterol may overwhelm the effect of androgens to prevent the accumulation of cholesterol and apoB-containing lipoproteins and thereby promote atherosclerotic lesion formation.

CONCLUSIONS

We have demonstrated that orchidectomy, which results in a marked reduction in testosterone, attenuates plasma cholesterol concentrations, apoB-containing lipoprotein formation, and atherosclerotic lesion formation in response to feeding a lowfat diet to mice with hyperlipidemia. These findings suggest that dietary constituents may have a profound influence on the modulation of lipid concentrations and atherogenesis by sex hormones.

Acknowledgments

The authors thank Eboni Lewis and Deborah Howatt for sharing their technical expertise. This work was funded by grants from the National Institutes of Health (P20RR021954 and HL082835 to Dr. King and HL82772 to Dr. Tannock). Drs. King and Tannock designed experiments, interpreted data, and wrote the manuscript. Dr. Zhang performed ovariectomies and orchidectomies. Dr. Hatch and Ms. Srodulski performed experiments and interpreted data. Drs. Chan and Zhang performed biological assays. Drs. Hatch, Chan, Zhang, and Ms. Srodulski all commented on the manuscript. Dr. Hatch and Ms. Srodulski contributed equally to this work.

REFERENCES

1. Roeters van Lennep JE, Westerveld HT, Erkelens DW, van der Wall EE. Risk factors for coronary heart disease: implications of gender. *Cardiovasc Res.* 2002; 53:538–549. [PubMed: 11861024]
2. Kolovou GD, Biliannou HG. Influence of aging and menopause on lipids and lipoproteins in women. *Angiology.* 2008; 59(Suppl):54S–57S. [PubMed: 18515273]
3. Xing D, Nozell S, Chen Y-F, et al. Estrogen and mechanisms of vascular protection. *Arterioscler Thromb Vasc Biol.* 2009; 29:289–295. [PubMed: 19221203]
4. Manson JE, Hsia J, Johnson KC, et al. Estrogen plus progestin and the risk of coronary heart disease. *N Engl J Med.* 2003; 349:523–534. [PubMed: 12904517]
5. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the women's health initiative randomized controlled trial. *JAMA.* 2002; 288:321–333. [PubMed: 12117397]
6. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *JAMA.* 1998; 280:605–613. [PubMed: 9718051]
7. Harman SM, Vittinghoff E, Brinton EA, et al. Timing and duration of menopausal hormone treatment may affect cardiovascular outcomes. *Am J Med.* 2011; 124:199–205. [PubMed: 21396500]
8. Bray PF, Larson JC, Lacroix AZ, et al. Usefulness of baseline lipids and c-reactive protein in women receiving menopausal hormone therapy as predictors of treatment-related coronary events. *Am J Cardiol.* 2008; 101:1599–1605. [PubMed: 18489937]
9. Makinen J, Jarvisalo MJ, Pollanen P, et al. Increased carotid atherosclerosis in andropausal middle-aged men. *J Am Coll Cardiol.* 2005; 45:1603–1608. [PubMed: 15893174]
10. Svartberg J, von Muhlen D, Mathiesen E, et al. Low testosterone levels are associated with carotid atherosclerosis in men. *J Intern Med.* 2006; 259:576–582. [PubMed: 16704558]
11. Makinen JJ, Perheentupa A, Irjala K, et al. Endogenous testosterone and serum lipids in middle-aged men. *Atherosclerosis.* 2008; 197:688–693. [PubMed: 17588587]
12. Yeap BB, Hyde Z, Almeida OP, et al. Lower testosterone levels predict incident stroke and transient ischemic attack in older men. *J Clin Endocrinol Metab.* 2009; 94:2353–2359. [PubMed: 19351733]
13. Hak AE, Witteman JC, de Jong FH, et al. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the rotterdam study. *J Clin Endocrinol Metab.* 2002; 87:3632–3639. [PubMed: 12161487]
14. Wu FC, von Eckardstein A. Androgens and coronary artery disease. *Endocr Rev.* 2003; 24:183–217. [PubMed: 12700179]
15. Kalyani RR, Dobs AS. Androgen deficiency, diabetes, and the metabolic syndrome in men. *Curr Opin Endocrinol Diabetes Obes.* 2007; 14:226–234. [PubMed: 17940444]
16. Bourghardt J, Wilhelmson AS, Alexanderson C, et al. Androgen receptor-dependent and independent atheroprotection by testosterone in male mice. *Endocrinology.* 2010; 151:5428–5437. [PubMed: 20861231]
17. Qiu Y, Yanase T, Hu H, et al. Dihydrotestosterone suppresses foam cell formation and attenuates atherosclerosis development. *Endocrinology.* 2010; 151:3307–3316. [PubMed: 20427482]
18. Hodgin JB, Maeda N. Minireview: estrogen and mouse models of atherosclerosis. *Endocrinology.* 2002; 143:4495–4501. [PubMed: 12446574]

19. Nettleship JE, Jones TH, Channer KS, Jones RD. Physiological testosterone replacement therapy attenuates fatty streak formation and improves high-density lipoprotein cholesterol in the tfm mouse: an effect that is independent of the classic androgen receptor. *Circulation*. 2007; 116:2427–2434. [PubMed: 17984376]
20. Alexandersen P, Haarbo J, Byrjalsen I, et al. Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits. *Circ Res*. 1999; 84:813–819. [PubMed: 10205149]
21. Nathan L, Shi W, Dinh H, et al. Testosterone inhibits early atherogenesis by conversion to estradiol: critical role of aromatase. *Proc Natl Acad Sci U S A*. 2001; 98:3589–3593. [PubMed: 11248122]
22. Mukherjee TK, Dinh H, Chaudhuri G, Nathan L. Testosterone attenuates expression of vascular cell adhesion molecule-1 by conversion to estradiol by aromatase in endothelial cells: implications in atherosclerosis. *Proc Natl Acad Sci U S A*. 2002; 99:4055–4060. [PubMed: 11904449]
23. Henriques TA, Huang J, D'Souza SS, et al. Orchidectomy, but not ovariectomy, regulates angiotensin ii-induced vascular diseases in apolipoprotein e-deficient mice. *Endocrinology*. 2004; 145:3866–3872. [PubMed: 15105380]
24. King VL, Hatch NW, Chan HW, et al. A murine model of obesity with accelerated atherosclerosis. *Obesity (Silver Spring)*. 2010; 18:35–41. [PubMed: 19498343]
25. King VL, Szilvassy SJ, Daugherty A. Interleukin-4 deficiency decreases atherosclerotic lesion formation in a site-specific manner in female ldl receptor^{-/-} mice. *Arterioscler Thromb Vasc Biol*. 2002; 22:456–461. [PubMed: 11884290]
26. Geary N, Asarian L, Korach KS, et al. Deficits in e2-dependent control of feeding, weight gain, and cholecystokinin satiation in er- α null mice. *Endocrinology*. 2001; 142:4751–4757. [PubMed: 11606440]
27. Wu J, Wang X, Chiba H, et al. Combined intervention of soy isoflavone and moderate exercise prevents body fat elevation and bone loss in ovariectomized mice. *Metabolism*. 2004; 53:942–948. [PubMed: 15254891]
28. Rogers NH, Perfield JW, Strissel KJ, et al. Reduced energy expenditure and increased inflammation are early events in the development of ovariectomy-induced obesity. *Endocrinology*. 2009; 150:2161–2168. [PubMed: 19179442]
29. Daly DM, Park SJ, Valinsky WC, Beyak MJ. Impaired intestinal afferent nerve satiety signalling and vagal afferent excitability in diet induced obesity in the mouse. *J Physiol*. 2011; 589:2857–2870. [PubMed: 21486762]
30. Gil-Ortega M, Stucchi P, Guzmán-Ruiz R, et al. Adaptative nitric oxide overproduction in perivascular adipose tissue during early diet-induced obesity. *Endocrinology*. 2010; 151:3299–3306. [PubMed: 20410199]
31. Shaul ME, Bennett G, Strissel KJ, et al. Dynamic, m2-like remodeling phenotypes of cd11c+ adipose tissue macrophages during high-fat diet-induced obesity in mice. *Diabetes*. 2010; 59:1171–1181. [PubMed: 20185806]
32. Freudenberger T, Oppermann M, Heim H-K, et al. Proatherogenic effects of estradiol in a model of accelerated atherosclerosis in ovariectomized apoe-deficient mice. *Basic Res Cardiol*. 2010; 105:479–486. [PubMed: 20177692]
33. Bourassa PA, Milos PM, Gaynor BJ, et al. Estrogen reduces atherosclerotic lesion development in apolipoprotein e-deficient mice. *Proc Natl Acad Sci U S A*. 1996; 93:10022–10027. [PubMed: 8816744]
34. Tsuda M, Iwai M, Li J-M, et al. Inhibitory effects of at1 receptor blocker, olmesartan, and estrogen on atherosclerosis via anti-oxidative stress. *Hypertension*. 2005; 45:545–551. [PubMed: 15723967]
35. Marsh MM, Walker VR, Curtiss LK, Banka CL. Protection against atherosclerosis by estrogen is independent of plasma cholesterol levels in ldl receptor-deficient mice. *J Lipid Res*. 1999; 40:893–900. [PubMed: 10224158]
36. Cazita PM, Berti JA, Aoki C, et al. Cholesteryl ester transfer protein expression attenuates atherosclerosis in ovariectomized mice. *J Lipid Res*. 2003; 44:33–40. [PubMed: 12518020]
37. Adams MR, Golden DL, Franke AA, et al. Dietary soy β -conglycinin (7s globulin) inhibits atherosclerosis in mice. *J Nutr*. 2004; 134:511–516. [PubMed: 14988439]

38. Elhage R, Arnal JF, Pieraggi MT, et al. 17 betaestradiol prevents fatty streak formation in apolipoprotein e-deficient mice. *Arterioscler Thromb Vasc Biol.* 1997; 17:2679–2684. [PubMed: 9409242]

Figure 1.

Effect of sex on plasma cholesterol and lipoprotein levels. (A) Total cholesterol was measured weekly for the first 4 weeks of diet ($n = 10$ per group). Lipoproteins were separated by fast protein liquid chromatography at (B) baseline, (C) Week 1 of diet, and (D) end of study. (E) Atherosclerotic lesion area was measured in the en face aorta after 17 weeks of following the diet; shown is mean (SEM) for $n = 6$ per group. * $P < 0.001$ females vs males; † $P < 0.0012$ females vs males.

Figure 2.

Effect of ovariectomy (ovx) in female mice. Female mice underwent ovx or sham operation (F-sham) at age 8 weeks ($n = 4-6$ per group), then fed the study diet starting 4 weeks later. (A) Uterine weight was decreased by ovx. OvX had no effect on (B) cholesterol or (C) triglyceride distribution on particles separated by fast protein liquid chromatography at study end. (D) OvX modestly attenuated atherosclerosis lesion development as measured in the en face aorta. * $P < 0.001$ sham vs ovx; † $P < 0.012$ ovx vs males; ‡ $P < 0.002$ males vs sham.

Figure 3.

Effect of orchidectomy (orx) on male mice. Male mice underwent orx or sham operation (M-sham) at age 8 weeks ($n = 5-11$ per group) then fed the study diet starting 4 weeks later. (A) Testosterone concentrations were decreased at week 17 in orx mice. Orx increased (B) cholesterol and (C) triglyceride distribution as analyzed by fast protein liquid chromatography. (D) Orx increased atherosclerotic lesion formation. * $P < 0.001$ sham vs females; † $P < 0.05$ sham vs females; ‡ $P < 0.001$ orx vs sham and females.

Table I

Mean (SEM) body weight of mice before and after 17 weeks of receiving the study diet

Group	Body Weight (g)	
	Beginning	Ending
Female - total	20.8 (0.5)	22.8 (0.2)
Male - total	27.9 (0.8)	28.9 (1.5)
Females - sham	20.6 (0.6)	24.6 (0.6)
Females - ovariectomized	20.7 (30.1)	30.1 (0.5)
Males - sham	26.3 (0.4)	29.9 (0.6)
Males - orchidectomized	24.5 (0.5)	26.6 (0.7)

Table II

Mean (SEM) lipid levels of mice after 17 weeks of receiving the study diet

Group	Cholesterol (mg/dL) *	Triglyceride (mg/dL) †
Female - total	657 (44)	133 (18)
Male - total	367 (54)	101 (8)
Female - sham	552 (20)	197 (13)
Female - ovariectomized	524 (22)	166 (17)
Male - sham	374 (30)	62 (10)
Male - orchidectomized	905 (35)	198 (22)

* To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.026. To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.6.
Cholesterol of 675 mg/dL = 17.1 mmol/L.

† To convert mg/dL triglyceride to mmol/L, multiply mg/dL by 0.0113. To convert mmol/L triglyceride to mg/dL, multiply mmol/L by 88.6.
Triglyceride of 133 mg/dL = 1.5 mmol/L.