Published in final edited form as:
Menopause. 2014 February ; 21(2): 143–152. doi:10.1097/GME.0b013e31829367c0.

Effects of Estradiol on Transcriptional Profiles in Atherosclerotic Iliac Arteries in Ovariectomized Cynomolgus Macaques

Kathleen M. Eyster, PhD[1,2], **Susan Appt, DVM**[3], **Abha Chalpe, PhD**[1], **Connie J. Mark-Kappeler, PhD**[1], **Thomas C. Register, PhD**[3], and **Thomas B. Clarkson, DVM**[3]

[1]Division of Basic Biomedical Sciences, Sanford School of Medicine of the University of South Dakota, Vermillion, SD 57069

[3]Department of Pathology (Comparative Medicine), Wake Forest University School of Medicine, Winston-Salem, NC 27157

Abstract

Objective—To assess the *in vivo* effects of estradiol treatment on arterial gene expression in atherosclerotic post-menopausal female monkeys.

Methods—Eight ovariectomized cynomolgus monkeys were fed atherogenic diets for 6.5 years. The left iliac artery was biopsied before randomization to estradiol (E2, 1 mg/day human equivalent dose, n=4) or vehicle (n=4) groups for 8 months. The right iliac artery was obtained at necropsy. Transcriptional profiles in pretreatment versus posttreatment iliac arteries were compared to assess responses of atherosclerotic arteries to estradiol.

Results—Iliac artery plaque size did not differ between E2 and placebo groups at baseline or over the treatment period. Nevertheless, estradiol treatment was associated with increased expression of 106 genes and decreased expression of 26 genes in iliac arteries. Estradiol treatment increased expression of extracellular matrix (ECM) genes, including types I and VI collagen (COL1A1, COL6A2) and fibulin-2 (FBLN-2), suggestive of an increase in the proportion or phenotype of smooth muscle or fibroblasts in the lesions. Also increased were components of the insulin-like growth factor (IGF) pathway [IGF-1, IGF binding protein 4 (IGFBP4) and IGFBP5], and the Wnt signaling pathway [secreted frizzled-related protein 2 (SFRP2), SFRP4, low density lipoprotein receptor-related protein 6 (LRP6), and Wnt 1 inducible signaling pathway protein 2 (WISP2)].

Conclusions—Estradiol treatment of monkeys with established atherosclerosis resulted in effects on iliac artery gene expression that suggested changes in the cellular composition of the lesions. Moreover, it is likely that the presence of atherosclerotic plaque affected the gene expression responses of the arteries to estrogen.

Keywords

estrogen; atherosclerosis; gene expression; microarray

[2]Corresponding author: Kathleen M. Eyster, Ph.D., Division of Basic Biomedical Sciences, Sanford School of Medicine of the University of South Dakota, 414 E. Clark St., Vermillion, SD 57069, Kathleen.Eyster@usd.edu phone: 605-677-5159 fax: 605-677-6381.

Conflicts of interest and sources of funding: The authors report no conflicts of interest in connection with the work described in this manuscript.

Introduction

The concept of atheroprotective effects of estradiol is supported by observational studies demonstrating that premenopausal women are less susceptible to most cardiovascular diseases than men of a similar age, but that the risk of cardiovascular disease increases following the menopause.[1, 2] Estradiol has been shown to have protective effects in early atherogenesis in a multitude of preclinical studies, but the protective effect is diminished in arteries that have already developed plaques.[3–6] Moreover, clinical trials suggest that there may be increased risk of cardiovascular disease events when treatment with estrogens and progestogens is initiated in women with pre-existing coronary heart disease[7] (HERS) and in older post-menopausal women.[8] Some evidence suggests that initiation of estrogen replacement at the time of the menopausal transition (when vasomotor symptoms and hot flushes are greatest) may produce greater beneficial effects on atherosclerosis than when administered in later menopause.[3] The loss of estradiol protection has been the source of much controversy in the discussion of the safety and efficacy of postmenopausal estrogen replacement, and the mechanism of the reversal remains obscure. Estrogen receptors alpha and beta are expressed in arterial tissues, thus direct effects of estrogen on arterial gene expression are likely.[9] Inverse relationships between ER expression and atherosclerosis have been observed at the protein[10] and mRNA level[11–13] in a variety of studies. It is possible that the loss of estrogen receptor expression in atherosclerotic arteries makes them refractive to direct estradiol effects. It is also likely that the complex cellular environment of the atherosclerotic lesion responds differently to estrogens than normal arteries or early, less complicated lesions.

Cynomolgus monkeys develop atherosclerotic lesions in peripheral (iliac) and coronary arteries after consuming a diet containing saturated fats and cholesterol in amounts similar to that consumed by people in North America. The ovariectomized cynomolgus monkey has been used extensively to study of the effects of estrogens on atherosclerosis in postmenopausal subjects.[14–16]

The current study was undertaken to examine the *in vivo* effects of oral estradiol on global gene expression patterns in iliac arteries from ovariectomized cynomolgus monkeys with established atherosclerosis.

Methods and Materials

Animals and Study Design

This study used 8 adult female surgically menopausal cynomolgus macaques *(Macaca fascicularis)* with an age range of 11–27 years. The monkeys had been ovariectomized for 4–6 years prior to initiation of the study. The animals had been housed in stable social groups of 3–4 animals each and consumed various semi-purified diets for 6.5 years. During the study, the monkeys continued to consume a typical North American-type diet which contained 0.20 mg cholesterol/Calorie, 29.6% fat, and 19.8% animal source protein (casein/lactalbumin). Monkeys were fed approximately 120 kcal/kg body weight once daily for the 8 months of the study.

All monkeys underwent an iliac biopsy procedure similar to that previously described[11, 12] to obtain pretreatment tissue samples (left common iliac artery). The iliac artery was divided into 3 equal segments (a, b, and c) from proximal to distal. Segment a was frozen in liquid nitrogen and stored at −70°C for a separate study. Segment b was placed flat on filter paper endothelial surface up, fixed in 4% paraformaldehyde for 24 hours and transferred to 70% ethanol and then embedded in paraffin for sectioning and staining of atherosclerotic plaque.

Measurement of plaque size was performed as previously described.[12, 17, 18] Segment c was cleaned of excess adventitia and placed in RNA later® (Sigma R-0901) for 24 hours and stored at −70°C prior to extraction of RNA.

Animals were randomized to control or estradiol treatment groups taking social groupings into consideration. Estradiol (Estrace®, 1mg tablets) was administered in the diet (n=4) at a women's equivalent dose of 1 mg/day, a dose commonly taken by postmenopausal women. Control animals received the same diet without hormone (n=4). After 8 months, all monkeys were euthanized and the contralateral (right) common iliac artery was collected and processed as described above.

Plasma Lipids and Lipoproteins

Blood samples were obtained at baseline and at 3, 6, and 8 months for the measurement of lipid levels.[18, 19] Plasma lipids/lipoproteins/total plasma cholesterol (TPC) concentrations, high-density lipoprotein cholesterol (HDL-C) concentrations, and plasma triglycerides (TGs) were determined in the Comparative Medicine Clinical Chemistry and Endocrinology Laboratory using reagents (ACE cholesterol, ACE HDL-C, and ACE triglycerides) and instrumentation (ACE ALERA autoanalyzer) from Alfa Wasserman Diagnostic Technologies (West Caldwell, NJ). TPC and HDL-C were standardized to calibrated controls from the Centers for Disease Control and Prevention-National Heart, Lung, and Blood Lipid Standardization Program. Intra- and inter-assay coefficients of variation were less than 5% for all analytes. Non-HDL-C, which approximates the sum of low-density lipoprotein (LDL) cholesterol and very-low-density lipoprotein (VLDL) cholesterol, was calculated by subtracting HDL-C from TPC.

All animal procedures conformed to State and Federal laws and were conducted in compliance with standards of the U.S. Department of Health and Human Services, and guidelines established by the Wake Forest University Animal Care and Use Committee (ACUC). The facilities and laboratory animal program of Wake Forest University are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

RNA extraction and DNA microarrays

Total RNA was extracted from the iliac arteries and analyzed as described.[11, 20] Analysis of gene expression in the cynomolgus monkey iliac arteries used CodeLink Whole Human Genome Bioarrays (Applied Microarrays, Tempe, AZ) as described.[11] For gene expression analysis, data from pretreatment iliac arteries were compared to posttreatment iliac arteries for both estradiol-treated animals and for vehicle control animals. Genes that differed in expression from baseline in both vehicle control and estradiol-treated lists were removed from further consideration.

Real time PCR

Two-step real time reverse transcription-polymerase chain reaction (PCR) was used to confirm expression of selected genes that had been shown to be differentially expressed by DNA microarray as described. [11] Complementary DNA (cDNA) was synthesized from 0.1 μg of total RNA by reverse transcription using the High Capacity cDNA Archive Kit from Applied Biosystems (Foster City, CA). The real time PCR reaction used cDNA from the reverse transcription reaction and thermal stable AmpliTaq Gold DNA polymerase for DNA amplification. Predesigned primers and probes were obtained from TaqMan Gene Expression Assays (Applied Biosystems) (insulin-like growth factor-1 (IGF1, Hs00153126_m1), insulin-like growth factor binding protein 4 (IGFBP4, Hs00181767_m1), IGFBP5 (Hs01052296_m1), secreted frizzled-related protein 4 (SFRP4, Hs00180066_m1),

secreted frizzled-related protein 2 (SFRP2, Hs00293258_m1), Wnt1 inducible signaling pathway protein 2 (WISP2, Hs00180242_m1), low density lipoprotein receptor-related protein 6 (LRP6, Hs00233945_m1). Glyceraldehyde phosphate dehydrogenase (GAPDH) was used as the housekeeping gene. Data from the real time PCR reactions were analyzed by qBase software.[21]

Statistical analysis

Unless specified otherwise, all data are expressed as the mean plus or minus the standard error of the mean (SE), the experimental number (n) was four per group, and the p value was set at 0.05. DNA microarrays: GeneSpring GX 7.0 software (Agilent, Santa Clara, CA) was used for statistical analysis of DNA microarray data. The p value was set at 0.05 for paired t test. The Benjamini and Hochberg False Discovery Rate of 0.05 was used for multiple testing correction. PCR data: Real time PCR data were analyzed by paired t-test followed by Newman-Keuls post hoc test when a significant F was found. Atherosclerotic lesion sizes were analyzed by ANOVA. Plasma lipid variables averaged over the treatment period were analyzed by ANCOVA using baseline values as a covariate. All parameters were normally distributed except for TG which was log transformed to improve normality.

Results

Analysis of gene expression in the monkey iliac arteries in response to estradiol in posttreatment arteries compared to pretreatment arteries, corrected for control, yielded a list of genes for which expression differed significantly with a p value of 0.05 or less. Criteria for exclusion from further analysis were: transcribed sequences with no name and no known function (because there is no current means to identify the functions of those genes), genes for which expression values were too near the limits of detection of the assay (average relative expression values less than 1.0), and genes for which the fold expression values were less than two-fold different from control (values between 0.5-fold and 2.0-fold of control values). The expression values of down-regulated genes ranged from 0.26–0.5-fold of control, whereas expression values of up-regulated genes ranged from 2 to 6.9-fold of control. Using these criteria, estradiol treatment resulted in differential expression of 132 genes (Table 1). Of those genes, 106 were up-regulated in response to estradiol and 26 were down-regulated.

For a subset of genes with evidence of increased expression in response to estradiol, microarray findings were followed up with real time RT-PCR analyses for verification purposes (Figure 1). Insulin-like growth factor 1 (IGF-1) was up-regulated by estradiol in the monkey iliac arteries as identified by DNA microarray analysis of gene expression (Figure 1A), and confirmed by RT-PCR (Figure 1B). Insulin-like growth factor binding proteins 4 (Figure 1C, D) and 5 (Figure 1E, F) were also up-regulated in the iliac arteries in response to estradiol. Several components of the Wnt signal transduction pathway were also up-regulated in the monkey iliac arteries in response to estradiol as initially shown by DNA microarray and confirmed by real time RT-PCR. These included secreted frizzled-related protein 2 (SFRP2) (Figure 2A, B), SFRP4 (Figure 2C, D), low density lipoprotein receptor-related protein 6 (LRP6) (Figure 2E, F), and Wnt-induced secreted protein 2 (WISP2, also called CCN5) (Figure 2G, H). A large number of other genes involved in signal transduction were represented in the estrogen regulated gene list, including connective tissue growth factor (CTGF), endoglin (ENG), and endothelial cell growth factor (ECGF1) (Table 1).

Several extracellular matrix genes were among those that were proportionally increased in the estrogen treated animals, including the alpha I chain of type I collagen (COL1A1), the alpha 2 chain of type VI collagen (COL6A2), fibulin (FBLN2), as well as others. The

transcript for heparan sulfate proteoglycan 2 (Perlecan) was also among the genes with significantly increased expression in the estrogen treated group (Table 1).

Atherosclerotic lesion sizes in pre- versus post-treatment comparisons or in vehicle versus estradiol treatment comparisons were not significantly different. In vehicle-treated control animals, iliac atherosclerotic plaque size at baseline averaged 0.36 ± 0.21 mm2 with a range of 0–0.87 mm2 (left iliac) while the right iliac collected at necropsy averaged 0.47±0.27 mm2, range 0.025–1.217 mm2. In the estradiol group, baseline plaque sizes averaged 0.34 ± 0.13 mm2 with a range of 0–0.61 mm2 (left iliac) while the right iliac collected post treatment averaged 0.79±0.31 mm2 with a range of 0–1.411 mm2. Plasma lipid levels were assessed at baseline and at 3, 6, and 8 months of the study. Triglyceride levels increased but no other significant differences in plasma lipids in response to estradiol treatment were identified (Table 2).

The data set for these DNA microarrays has been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) as recommended by Minimum Information About a Microarray Experiment (MIAME) standards[22] and can be accessed through GEO Series accession numbers GSE37187 and GSE26326 (GSM646190, GSM646191, GSM646194, GSM646195, and GSM646186-9).

Discussion

The treatment paradigm used in this study was designed to mimic the effects of estradiol treatment of postmenopausal women initiated after a long period of estrogen deficiency. The power of this experimental design is that it provides “before” and “after” snapshots of gene expression in atherosclerotic iliac arteries in response to estradiol. Moreover, each animal served as its own control in this experimental design which provided an internal control for atherosclerosis that would not be practical or possible in a human population. As in women, there was considerable heterogeneity in the presence and size of atherosclerotic plaque at the time of initiation of estradiol treatment. We and others[11, 23, 24] have shown that the presence of atherosclerosis dramatically alters gene expression in the arteries, which may originate in part from the variety of cell types present in an atherosclerotic lesion.[12, 13, 25] The variance in gene expression due to the influence of atherosclerosis and multiple cell types present may have masked subtle responses to estrogen, or may have inflated other estrogen responses.

These data demonstrate a substantial effect of estrogen on gene expression in the iliac arteries despite the minimal effect of estrogen on plasma lipid levels and lack of effect on atherosclerotic plaque size. The data are consistent with previous reports of a limited effect of estrogen on lipid levels[14, 18] and inability of estrogen treatment to reduce atherosclerotic plaque size in well-established atherosclerosis[3, 27, 28] in cynomolgous monkeys. However, the presence of genomic estrogen receptors in the vasculature is well established.[29] Indeed, estrogen has been shown to affect a broad scope of receptor-mediated physiological functions in the vasculature, ranging from angiogenesis to reductions in vascular tone, modulation of inflammatory reactions, and other functions.[5, 6] Since genomic estrogen receptors act as ligand-activated transcription factors,[30] it is logical to examine the effects of estrogen on gene expression in the arteries in spite of the limited effects of estrogen on lipid levels or atherosclerotic plaque size.

The most important limitation of this study is the relatively small number of subjects which were assessed for global gene expression analyses as necessitated by the expense of the model. Since the presence of complicated plaque with a variety of unique cell types (endothelial, smooth muscle, macrophage, fibroblast, T cell, B cell, etc.) has such a

profound effect on the expression profile of the whole tissue,[11] it is likely that the presence of plaque at least partially masked the effects of estradiol on gene expression. Thus, genes that were observed to respond to estradiol in the presence of an environment compromised by the presence of atherosclerosis represent a combination of effects of estrogen on individual phenotypes as well as an alteration in the populations of those cell types. Unfortunately, there was not enough statistical power in the group sizes to dissect out the effects of estradiol on gene expression in arteries with small/absent plaque versus medium or large plaque. Some genes that have been previously shown to be regulated by estradiol in the vasculature[31–33] were not identified in this study; we hypothesize that the presence of atherosclerotic plaque in the arteries is responsible, at least in part, for this discrepancy. Other possible explanations for the discrepancy include different sensitivity of methods used in this versus other experiments, different lengths of exposure to hormone, different statistics (e.g., multiple testing correction may eliminate genes whose expression is actually different), or different responses to hormone in different vascular beds.

Several of the expression profile changes in response to estrogen suggest that there were profound effects on cell phenotypes and/or cell populations present in the artery. For example, two to four fold increases in the expression of extracellular matrix genes such as types I and VI collagen are more representative of a smooth muscle or fibroblastic phenotype than macrophage phenotype. Indeed, the ontology of extracellular matrix was one of the few ontologies in which all of the differentially expressed genes in response to estrogen were up-regulated. These data suggest that there may be some changes in arterial cell populations in response to estrogen. Fibulin-2, an extracellular matrix protein that is elevated in smooth muscle rich regions of atherosclerotic plaques, was increased by nearly 3 fold in the estradiol treated group. Fibulin-2 is a participant in smooth muscle cell migration via interactions with other extracellular matrix molecules such as hyaluronic acid and versican.[34] These data again suggest an enhancement of smooth muscle cell phenotypes in the lesion. The transcript for heparan sulfate proteoglycan 2 (Perlecan) was also among the genes with significantly increased expression in the estrogen treated group. Perlecan is also associated with the smooth muscle cell phenotype and its increase in this model is consistent with observations in non-atherosclerotic castrated male rat carotid arteries.[35]

Two signaling pathways were chosen for confirmation of gene expression by real time RT-PCR. These gene families were chosen because multiple members of the pathway were up-regulated in response to estrogen in both cases. Components of the IGF-1 signaling pathway were chosen for follow-up studies because a significant body of literature exists on this pathway and its estrogen-responsiveness. The data reported here will add to that literature. The Wnt signaling pathway was chosen for follow-up confirmatory studies for the opposite reason. Although multiple components of the Wnt pathway were up-regulated in the iliac arteries in response to estrogen, only a minimal amount of information was found in the literature on the potential role of this pathway in the vasculature[36, 37] and no information on its estrogen response in the arteries.

Insulin-like growth factor I (IGF-I) gene expression was increased in the monkey iliac artery in response to estradiol. Estradiol has been shown to increase IGF-I levels in other tissues such as uterine smooth muscle.[38] In contrast to the current data, we have shown that IGF-1 decreased in the rat mesenteric arteries in response to ethinyl estradiol.[39] Similarly, Scheidegger and coworkers[40] showed that estrogen caused a decrease in IGF-1 expression in aortic smooth muscle cells. These data thus indicate a pleiotropic effect of estrogen on IGF-1 in the vasculature. The differing responses of IGF-1 to estrogen may be due to differences in vascular bed,[41] or to the presence of atherosclerotic plaque in the monkey iliac arteries compared to rat mesenteric arteries or cultured aortic smooth muscle cells. IGF-I itself has pleiotropic effects in the vasculature.[42] IGF-I caused vasorelaxation in

normotensive but not hypertensive rats,[43] and IGF-I has been reported to have atheroprotective[44] and well as atherogenic[45] effects which may depend on the system and modulation by IGF binding proteins. It is intriguing to speculate whether some of the pleiotropic effects of estrogen in the vasculature are mediated by IGF-I since its effects are also pleiotropic. IGF binding proteins transport IGF and typically inhibit access of the growth factor to its receptor.[46] Increased expression of IGF1 as well as IGFBP4 and IGFBP5, as shown in this study, appears antithetical; further research will be required to determine which of these factors may mediate positive versus negative effects of estrogen in the arteries.

Four genes related to the Wnt signal transduction pathway were up-regulated in the monkey iliac arteries in response to estradiol. Activation of the Wnt pathway has been reported to increase monocyte adhesion to endothelial cells,[47] a critical process in the development of atherosclerosis. SFRP2 and SFRP4 are inhibitors of the Wnt pathway; they are secreted soluble proteins that bind the ligand, Wnt, and prevent its binding to the receptor, called frizzled.[47] In this context, up-regulation of SFRP2 and SFRP4 may mediate protective effects of estradiol on the arteries. On the other hand, we have observed a down-regulation of SFRP4 in response to ethinyl estradiol and equilin in the rat mesenteric arteries,[39] in a different species, a different vascular bed, and in the absence of atherosclerosis. In contrast, LRP6 is a co-receptor for frizzled.[49] Up-regulation of LRP6 suggests increased sensitivity of the Wnt pathway to ligand activation which seems contradictory to the up-regulation of inhibitors SFRP2 and SFRP4 described above. WISP2 (also called CCN5) expression is increased in response to activation of the Wnt signaling pathway,[50] although its expression has also been shown to increase in response to estrogen[51] and IGF-1[52] in other tissues. CCN proteins are cysteine-rich, modular proteins named for the first 3 proteins found to contain the modules.[53] CCN proteins, including WISP2/CCN5, are involved in adhesion, mitosis, extracellular matrix production, migration, and IGF binding.[53] Thus WISP2/CCN5 may form a nexus of signaling for Wnt, estrogen, and IGF1 in the arteries. Additional research will be required to identify the specific role of WISP2/CCN5, as well as the other Wnt pathway components, in the vasculature.

Summary and Conclusions

The effects of estradiol on gene expression in monkey iliac arteries reported herein may reflect alterations in both proportions of individual cell populations as well as modulation of individual phenotype and gene expression within those cell populations that occurred during the eight months of treatment with estrogen. In turn, individual cell population responses are likely to be different in a complex lesion of cellular heterogeneity than in purified cell populations in vitro or in a normal artery. Overall the results suggest a shift towards extracellular matrix production phenotypes reflecting smooth muscle and fibroblastic contributions to arterial biology. Moreover, it is likely that the presence of atherosclerotic plaque as well as the heterogeneity of those plaques affected the gene expression responses of the arteries to estrogen. Little to no evidence for increased arterial inflammation in response to estradiol in these atherosclerotic arteries was observed.

Acknowledgments

This work was supported by PO1 HL45666 (TBC), RO1 AG28641 (TCR), and RO1 AG27847 (SEA). DNA microarray and real time RT-PCR experiments were carried out in the Genomics Core facility at the University of South Dakota which is supported by NIH INBRE 2 P20 RR016479.

References

1. Calhoun DA, Oparil S. Gender and blood pressure. Hypertension Primer (2nd Edition). 1999:229–232.
2. Mendelsohn ME. Mechanisms of estrogen action in the cardiovascular system. J Steroid Biochem Molec Biol. 2000; 74:337–343. 2000. [PubMed: 11162942]
3. Clarkson TB. Estrogen effects on arteries vary with stage of reproductive life and extent of subclinical atherosclerosis progression. Menopause. 2007; 14:373–384. [PubMed: 17438515]
4. Dubey RK, Imthurn B, Barton M, Jackson EK. Vascular consequences of menopause and hormone therapy: Importance of timing of treatment and type of estrogen. Cardiovasc Res. 2005; 66:295–306. [PubMed: 15820198]
5. Miller VM, Duckles SP. Vascular actions of estrogens: functional implications. Pharmacol Rev. 2008; 60:210–241. [PubMed: 18579753]
6. Xing D, Nozell S, Chen Y-F, Hage F, Oparil S. Estrogen and mechanisms of vascular protection. Arterioscler Thromb Vasc Biol. 2009; 29:289–295. [PubMed: 19221203]
7. 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]
8. Writing group for the Women's Health Initiative investigators. Risks and benefits of estrogen plus progestin in healthy post-menopausal women. Principal results from the Women's Health Initiative randomized controlled trial. JAMA. 2002; 288:321–333. [PubMed: 12117397]
9. Register TC, Adams MR. Coronary artery and cultured aortic smooth muscle cells express mRNA for both the classical estrogen receptor and the newly described estrogen receptor beta. J Steroid Biochem Mol Biol. 1998; 64(3–4):187–191. [PubMed: 9605413]
10. Losordo DW, Kearney M, Kim EA, Jekanowski J, Isner JM. Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries of premenopausal women. Circulation. 1994; 89:1501–1510. [PubMed: 8149515]
11. Eyster KM, Appt S, Mark-Kappeler CJ, Chalpe A, Register T, Clarkson TB. Gene expression signatures differ with extent of atherosclerosis in monkey iliac artery. Menopause. 2011; 18:1087–1095. 2011. [PubMed: 21646924]
12. Walker SE, Register TC, Appt SE, et al. Plasma lipid-dependent and -independent effects of dietary soy protein and social status on atherogenesis in premenopausal monkeys: implications for postmenopausal atherosclerosis burden. Menopause. 2008; 15:950–957. [PubMed: 18427358]
13. Register TC. Primate models in women's health: inflammation and atherogenesis in female cynomolgus macaques (Macaca fascicularis). Am J Primatol. 2009; 71(9):766–775. [PubMed: 19530126]
14. Clarkson TB, Anthony MS, Morgan TM. Inhibition of postmenopausal atherosclerosis progression: a comparison of the effects of conjugated equine estrogens and soy phytoestrogens. J Clin Endocrinol Metab. 2001; 86:41–47. [PubMed: 11231976]
15. Corner GW. Ovulation and menstruation in Macacus rhesus. Contrib Embryol. 1923; 15:73–102.
16. Gilardi KV, Shideler SE, Valverde CR, Roberts JA, Lasley BL. Characterization of the onset of menopause in the rhesus macaque. Biol Reprod. 1997; 57:335–340. [PubMed: 9241047]
17. Clarkson, TB. Progression and regression of nonhuman primate coronary artery atherosclerosis: considerations of experimental design. In: Malinow, MR.; Blaton, VH., editors. Regression of atherosclerotic lesions. Vol. 79. New York: Plenum Press; NATO ASI Series A; 1984. p. 43-60.
18. Clarkson TB, Ethun KF, Chen H, Golden D, Floyd E, Appt SE. Effects of bazedoxifene alone and with conjugated equine estrogens on coronary and peripheral artery atherosclerosis in postmenopausal monkeys. Menopause. 2013; 30:274–281. [PubMed: 23435024]
19. Adams MR, Golden DL, Williams JK, Franke AA, Register TC, Kaplan JR. Soy protein containing isoflavones reduces the size of atherosclerotic plaques without affecting coronary artery reactivity in adult male monkeys. J Nutr. 2005; 135:2852–2856. [PubMed: 16317131]
20. Eyster KM, Klinkova O, Kennedy V, Hansen KA. Whole genome deoxyribonucleic acid microarray analysis of gene expression in ectopic versus eutopic endometrium. Fertil Steril. 2007; 88:1505–1533. [PubMed: 17462640]

21. Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 2007; 8:R19. [PubMed: 17291332]
22. Brazma A, Hingamp P, Quackenbush J, et al. Minimum information about a microarray experiment (MIAME)—toward standards for microarray data. Nature Genetics. 2001; 29:365–371. [PubMed: 11726920]
23. Lutgens E, Faber B, Schapira K, et al. Gene profiling in atherosclerosis reveals a key role for small inducible cytokines. Validation using a novel monocyte chemoattractant protein monoclonal antibody. Circulation. 2005; 111:3443–3452. [PubMed: 15967845]
24. Bijnens AP, Lutgens E, Ayoubi T, Kuiper J, Horrevoets AJ, Daemen MJ. Genome-wide expression studies of atherosclerosis. Critical issues in methodology, analysis, interpretation of transcriptomics data. Arterioscler Thromb Vasc Biol. 2006; 26:1226–1235. [PubMed: 16574897]
25. Walker SE, Adams MR, Franke AA, Register TC. Effects of dietary soy protein on iliac and carotid artery atherosclerosis and gene expression in male monkeys. Atherosclerosis. 2008; 196:106–113. [PubMed: 17367795]
26. Shelton, KA.; Clarkson, TB.; Kaplan, JR. Nonhuman primate models of atherosclerosis. In: Abee, CR.; Mansfield, K.; Tardif, SD.; Morris, T., editors. Nonhuman Primates in Biomedical Research. 2nd ed.. San Diego, CA: Elsevier; 2012. p. 385-411.
27. Clarkson TB. The new conundrum: do estrogens have any cardiovascular benefits. Int J Fertil. 2002; 47:61–68.
28. Williams JK, Anthony MS, Honore EK, et al. Regression of atherosclerosis in female monkeys. Arterioscler Thromb Vasc Biol. 1995; 15:827–836. [PubMed: 7600113]
29. Karas RH, Patterson BL, Mendelsohn ME. Human vascular smooth muscle cells contain functional estrogen receptor. Circulation. 1994; 89:1943–1950. [PubMed: 8181116]
30. Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. J Biol Chem. 2001; 276:36869–36872. [PubMed: 11459850]
31. Egan KM, Lawson JA, Fries S, et al. COX-2 derived prostacyclin confers atheroprotection on female mice. Science. 2004; 306:1954–1957. [PubMed: 15550624]
32. Lewandowski KC, Komorowski J, Mikhalidis DP, et al. Effects of hormone replacement therapy type and route of administration on plasma matrix metalloproteinases and their tissue inhibitors in postmenopausal women. J Clin Endocrinol Metab. 2006; 91:3123–3130. [PubMed: 16705077]
33. Yang S, Bae L, Zhang L. Estrogen increases eNOS and Nox release in human coronary artery endothelium. J Cardiovasc Pharm. 2000; 36:242–247.
34. Ström A, Olin AI, Aspberg A, Hultgårdh-Nilsson A. Fibulin-2 is present in murine vascular lesions and is important for smooth muscle cell migration. Cardiovasc Res. 2006; 69(3):755–763. [PubMed: 16409997]
35. Pompei LM, Steiner ML, Theodoro TR, et al. Effect of estrogen therapy on vascular perlecan and metalloproteinases 2 and 9 in castrated rats. Climacteric. 2013; 16:147–153. [PubMed: 22640525]
36. Goodwin AM, D'Amore PA. Wnt signaling in the vasculature. Angiogenesis. 2002; 5:1–9. [PubMed: 12549854]
37. Zerlin M, Julius MA, Kitajewski J. Wnt/frizzled signaling in angiogenesis. Angiogenesis. 2008; 11:63–69. [PubMed: 18253847]
38. Swartz CD, Afshari CA, Yu L, Hall KE, Dixon D. Estrogen-induced changes in IGF-I, Myb family, and MAP kinase pathway genes in human uterine leiomyoma and normal uterine smooth muscle cell lines. Mol Hum Reprod. 2005; 11:441–450. [PubMed: 15879465]
39. Mark-Kappeler C, Martin DS, Eyster KM2011. Estrogens and selective estrogen receptor modulators regulate gene and protein expression in the mesenteric arteries. Vasc Pharm. 2011; 55:42–49.
40. Scheidegger KJ, Cenni B, Picard D, Delafontaine P. Estradiol decreases IGF-1 and IGF-1 receptor expression in rat aortic smooth muscle cells. Mechanisms for its atheroprotective effects. J Biol Chem. 2000; 275:38921–38928. [PubMed: 10982795]
41. Zhang J, Burridge KA, Friedman MH. In vivo differences between endothelial transcriptional profiles of coronary and iliac arteries revealed by microarray analysis. Am J Physiol Heart Circ Physiol. 2008; 295:H1556–H1561. [PubMed: 18689496]

42. Conti E, Carrozza C, Capoluongo E, et al. Insulin-like growth factor-I as a vascular protective factor. Circulation. 2004; 110:2260–2265. [PubMed: 15477425]
43. McCallum RW, Hamilton CA, Graham D, Jardine E, Connel JMC, Dominiczak AF. Vascular responses to IGF-I and insulin are impaired in aortae of hypertensive rats. J Hypertension. 2005; 23:351–358.
44. Higashi Y, Sukhanov S, Anwar A, Shai SY, Delafontaine P. Aging, atherosclerosis, and IGF-1. J Gerontol A Biol Sci Med Sci. 2012; 67(6):626–639. [PubMed: 22491965]
45. Bayes-Genis A, Conover CA, Schwartz RS. The insulin-like growth factor axis: a review of atherosclerosis and restenosis. Circ Res. 2000; 86:125–130. [PubMed: 10666406]
46. Delafontaine P, Song Y-H, Li Y. Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels. Arterioscler Thromb Vasc Biol. 2004; 24:435–444. [PubMed: 14604834]
47. Lee DK, Grantham RN, Trachte AL, Mannion JD, Wilson CL. Activation of the canonical Wnt/β-catenin pathway enhances monocyte adhesion to endothelial cells. Biochem Biophys Res Comm. 2006; 347:109–116. [PubMed: 16815294]
48. Kawano Y, Kypta R. Secreted antagonists of the Wnt signalling pathway. J Cell Sci. 2003; 116:2627–2634. [PubMed: 12775774]
49. He X, Semenov M, Tamai K, Zeng X. LDL receptor-related proteins 5 and 6 in Wnt/β-catenin signaling: arrows point the way. Development. 2004; 131:1663–1677. [PubMed: 15084453]
50. Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, et al. Wnt/beta-catenin signaling is a normal physiological response to mechanical loading in bone. J Biol Chem. 2006; 281:31720–31728. [PubMed: 16908522]
51. Inadera H, Hashimoto S, Dong HY, et al. WISP-2 as a novel estrogen-responsive gene in human breast cancer cells. Biochem Biophys Res Commun. 2000; 275:108–114. [PubMed: 10944450]
52. Dhar K, Banerjee S, Dhar G, Sengupta K, Banerjee SK. Insulin-like growth factor-1 (IGF-1) induces WISP2/CCN5 via multiple molecular cross-talks and is essential for mitogenic switch by IGF-1 axis in estrogen receptor-positive breast tumor cells. Cancer Res. 2007; 67:1520–1526. [PubMed: 17308090]
53. Brigstock DR. The CCN family: a new stimulus package. J Endocrinol. 2003; 178:169–175. [PubMed: 12904165]

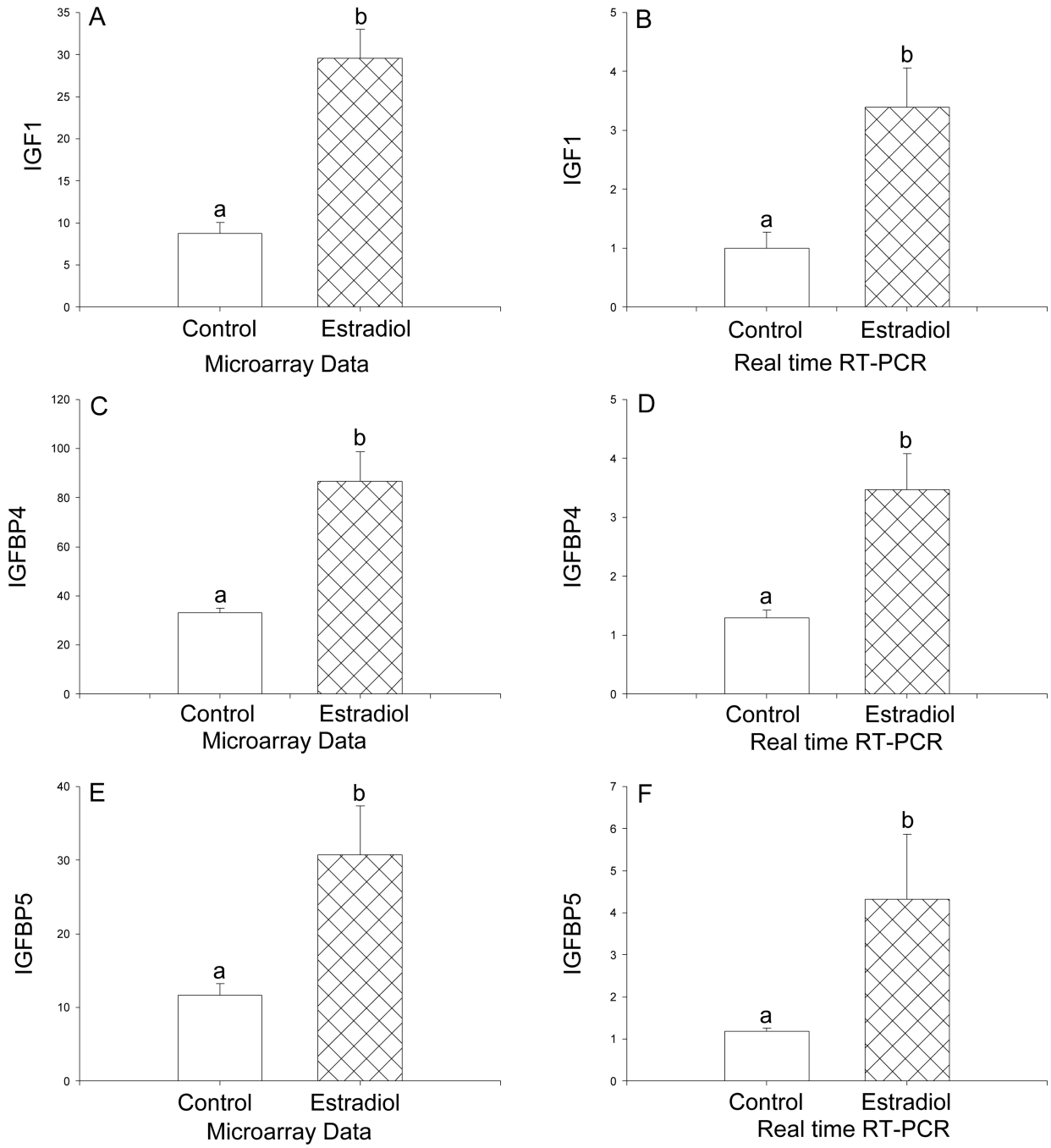

Figure 1.

Differential expression of insulin-like growth factor 1 (IGF-1) (A, B), insulin-like growth factor binding protein 4 (IGFBP4) (C, D), and insulin-like growth factor binding protein 5 (IGFBP5) (E, F) in the iliac arteries of cynomolgus monkeys fed a high fat/high cholesterol diet in response to treatment with estradiol. Panels A, C, E: Differential gene expression data from DNA microarray analysis. Panels B, D, F: Data from real time RT-PCR analysis. Data are expressed as mean ± SEM, n=4/group. Bars with different letter superscripts denote that the data for those groups are significantly different from each other, $p<0.05$.

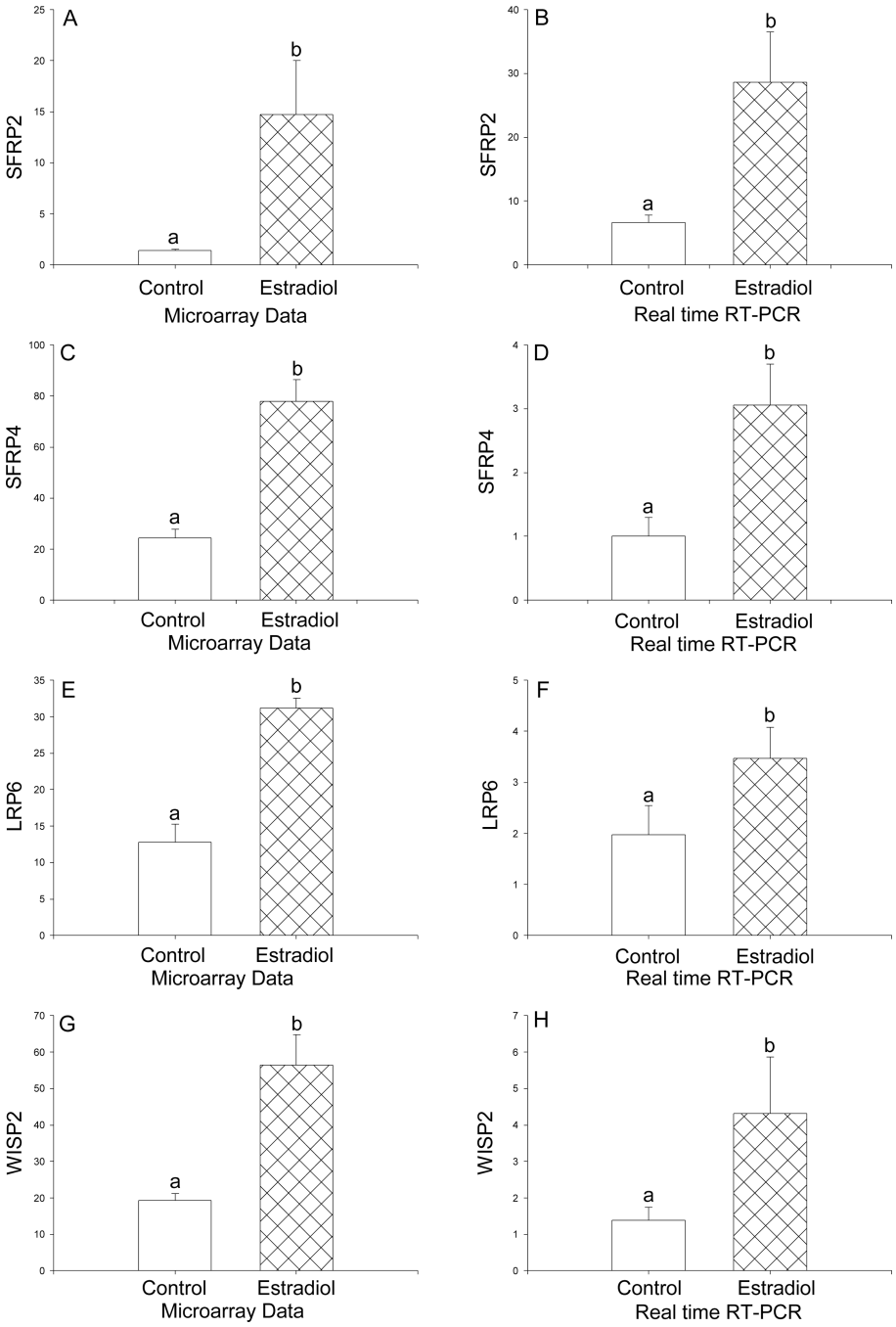

Figure 2.

Differential expression of secreted frizzled-related protein 2 (SFRP2) (A, B), secreted frizzled-related protein 4 (SFRP4) (C, D), low density lipoprotein receptor-related protein 6 (LRP6) (E, F), and Wnt-induced secreted protein 2 (WISP2, also called CCN5) (G, H) in the iliac arteries of cynomolgus monkeys fed a high fat/high cholesterol diet in response to treatment with estradiol. Panels A, C, E, G: Data from DNA microarray analysis. Panels B, D, F, H: Data from real time RT-PCR analysis. Data are expressed as the mean ± SEM, n=4/group. Statistical analysis utilized Student's t-test. Bars with different letter superscripts denote that the data for those groups are significantly different from each other, $p<0.05$.

Table 1

Differentially expressed genes in the iliac arteries of ovariectomized cynomolgus macaques treated with estradiol for 8 months. Gene expression values are shown for pretreatment baseline data (PreTrt; n=4) and for post estradiol treatment (PostTrt; n=4). Fold expression data (Fold) compare posttreatment to pretreatment values for each gene (PostTrt/PreTrt). The GenBank accession number (GenBank ACCN #) and p value for statistical significance (p val) are also shown.

Genbank ACCN#	Gene Name	PreTrt	PostTrt	Fold	p val
	Signal transduction				
NM_005737	ADP-ribosylation factor-like 7 (ARL7)	0.37	1.37	3.7	0.017
NM_005184	calmodulin 3 (phosphorylase kinase, delta) (CALM3)	1.10	2.53	2.3	0.011
NM_052854	cAMP responsive element binding protein 3-like 1 (CREB3L1)	0.71	1.61	2.3	0.007
NM_020404	CD164 sialomucin-like 1 (CD164L1)	5.35	16.35	3.0	0.007
NM_001826	CDC28 protein kinase regulatory subunit 1B (CKS1B)	7.85	3.63	0.5	0.024
NM_015424	chordin-like 2 (CHRDL2)	1.81	3.65	2.0	0.005
NM_001901	connective tissue growth factor (CTGF)	5.08	2.60	0.5	0.043
NM_004717	diacylglycerol kinase, iota (DGKI)	5.39	2.46	0.5	0.010
NM_000118	endoglin (ENG)	2.71	6.91	2.5	0.026
NM_001953	endothelial cell growth factor 1 (ECGF1)[a]	0.68	3.03	4.5	0.019
BC036034	endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor 2	2.93	1.40	0.5	0.020
NM_014597	estrogen receptor binding protein (ERBP)	18.20	9.04	0.5	0.0001
NM_007369	G protein-coupled receptor 161 (GPR161)	5.37	12.83	2.4	0.001
NM_004838	homer homolog 3 (HOMER3)	0.51	1.35	2.6	0.021
NM_000618	insulin-like growth factor 1 (somatomedin C) (IGF1)	7.01	29.54	4.2	0.0001
NM_001552	insulin-like growth factor binding protein 4 (IGFBP4)	33.09	86.56	2.6	0.004
NM_000599	insulin-like growth factor binding protein 5 (IGFBP5)	11.63	30.70	2.6	0.010
NM_002336	low density lipoprotein receptor-related protein 6 (LRP6)	12.76	31.21	2.4	0.012
NM_005456	mitogen-activated protein kinase 8 interacting protein 1 (MAPK8IP1)	1.51	3.84	2.5	0.006
NM_015093	mitogen-activated protein kinase kinase kinase 7 interacting protein 2 (MAP3K7IP2)	18.84	9.62	0.5	0.003
NM_006184	nucleobindin 1 (NUCB1)	11.06	31.61	2.9	0.006
NM_002548	olfactory receptor, family 1, subfamily D, member 2	5.64	11.35	2.0	0.001

Genbank ACCN#	Gene Name	PreTrt	PostTrt	Fold	p val
	Signal transduction				
	(OR1D2)				
NM_005866	opioid receptor, sigma 1 (OPRS1)	3.55	7.75	2.2	0.004
NM_198964	parathyroid hormone-like hormone (PTHLH)	12.48	25.79	2.1	0.022
AF318382	pp9974 mRNA [Insulin-like Growth Factor 2]	38.03	107.20	2.8	0.004
NM_014225	protein phosphatase 2, regulatory subunit A (PR 65), alpha (PPP2R1A)	12.48	27.05	2.2	0.001
NM_006266	ral guanine nucleotide dissociation stimulator (RALGDS)[a]	4.18	8.60	2.1	0.046
NM_024832	Ras and Rab interactor 3 (RIN3)	0.96	2.04	2.1	0.022
NM_198230	regulator of G-protein signalling 12 (RGS12)	3.37	6.68	2.0	0.005
NM_017790	regulator of G-protein signalling 3 (RGS3)	2.28	5.86	2.6	0.040
NM_006744	retinol binding protein 4, plasma (RBP4)	0.76	2.38	3.1	0.009
NM_004309	Rho GDP dissociation inhibitor (GDI) alpha (ARHGDIA)	12.73	26.99	2.1	0.042
NM_003013	secreted frizzled-related protein 2 (SFRP2)	6.58	28.66	4.4	0.006
NM_003014	secreted frizzled-related protein 4 (SFRP4)	26.48	77.97	2.9	0.001
NM_006747	signal-induced proliferation-associated gene 1 (SIPA1)[a]	3.43	7.86	2.3	0.045
BC041395	similar to diaphanous homolog 3[a]	1.25	0.44	0.4	0.012
NM_145245	similar to ecotropic viral integration site 5	2.52	6.69	2.7	0.001
AK124904	similar to Rho/Rac guanine nucleotide exchange factor	3.65	1.81	0.5	0.007
N22508	similar to tumor necrosis factor receptor 2	2.71	6.67	2.5	0.004
NM_182759	TAFA3 protein (TAFA3)	3.15	7.93	2.5	0.006
NM_031937	TBC1 domain family, member 10 (TBC1D10)	2.20	4.36	2.0	0.017
NM_016151	thousand and one amino acid protein kinase (TAO1)	5.54	13.54	2.4	0.001
NM_003271	transmembrane 4 superfamily member 7 (TM4SF7)[a]	4.10	12.62	3.1	0.028
NM_003390	WEE1 homolog (WEE1)	3.84	1.70	0.4	0.008
NM_030798	Williams-Beuren syndrome chromosome region 16 (WBSCR16)	0.78	1.60	2.1	0.036
NM_000377	Wiskott-Aldrich syndrome (eczema-thrombocytopenia) (WAS)	6.25	14.87	2.4	0.037
NM_003881	WNT1 inducible signaling pathway protein 2 (WISP2)	19.26	56.42	2.9	0.001

Genbank ACCN#	Gene Name	PreTrt	PostTrt	Fold	p val
	Signal transduction				
NM_012478	WW domain binding protein 2 (WBP2)	6.83	14.68	2.1	0.049
	Cytoskeleton				
NM_005718	actin related protein 2/3 complex, subunit 4, 20kDa (ARPC4)	0.48	1.06	2.2	0.040
NM_015033	formin binding protein 1 (FNBP1)	17.44	38.65	2.2	0.017
NM_006932	smoothelin (SMTN)[a]	29.97	70.72	2.4	0.046
AB041269	keratin 19	0.49	1.18	2.4	0.008
NM_016140	brain specific protein (CGI-38) [tubulin polymerization-promoting protein 3]	4.25	14.24	3.4	0.038
	Defense/Immune system				
NM_030968	C1q and tumor necrosis factor related protein 1 (C1QTNF1)	39.98	79.19	2.0	0.023
NM_004887	chemokine (C-X-C motif) ligand 14 (CXCL14)	1.55	8.32	5.4	0.0001
NM_005218	defensin, beta 1 (DEFB1)	0.40	2.09	5.2	0.003
NM_024626	immune costimulatory protein B7-H4 (B7-H4)	20.73	41.90	2.0	0.002
NM_052868	immunoglobulin superfamily, member 8 (IGSF8)	4.94	10.99	2.2	0.001
NM_021034	interferon induced transmembrane protein 3 (1-8U) (IFITM3)	47.79	103.63	2.2	0.022
NM_006863	leukocyte immunoglobulin-like receptor A1 (with TM domain) (LILRA1)	2.85	5.82	2.0	0.009
NM_002346	lymphocyte antigen 6 complex, locus E (LY6E)	7.26	20.16	2.8	0.002
NM_009588	lymphotoxin beta (TNF superfamily, member 3) (LTB)	1.37	3.60	2.6	0.001
NM_005516	major histocompatibility complex, class I, E (HLA-E)[a]	22.45	55.43	2.5	0.048
	Transcriptional regulation				
NM_001206	basic transcription element binding protein 1 (BTEB1)	0.57	1.59	2.8	0.005
NM_004779	CCR4-NOT transcription complex, subunit 8 (CNOT8)	7.99	3.85	0.5	0.0001
NM_001453	forkhead box C1 (FOXC1)	38.48	78.88	2.1	0.041
NM_004118	forkhead-like 18 (FKHL18)	3.11	10.42	3.4	0.031
NM_199072	I-mfa domain-containing protein (HIC)	10.20	2.98	0.3	0.015
NM_002167	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein (ID3)	5.00	19.14	3.8	0.018

Menopause. Author manuscript; available in PMC 2015 February 01.

Genbank ACCN#	Gene Name	PreTrt	PostTrt	Fold	p val
	Signal transduction				
NM_015995	Kruppel-like factor 13 (KLF13)	12.30	29.84	2.4	0.001
NM_012317	leucine zipper, down-regulated in cancer 1 (LDOC1)	9.05	22.00	2.4	0.013
NM_002653	paired-like homeodomain transcription factor 1 (PITX1)	2.15	5.66	2.6	0.040
NM_003120	spleen focus forming virus (SFFV) proviral integration oncogene spi1 (SPI1)	1.38	4.86	3.5	0.050
NM_004236	thyroid receptor interacting protein 15 (TRIP15)	43.69	20.80	0.5	0.001
NM_031283	transcription factor 7-like 1 (T-cell specific, HMG-box) (TCF7L1)	4.87	10.25	2.1	0.043
NM_014112	trichorhinophalangeal syndrome I (TRPS1)	29.80	13.70	0.5	0.013
NM_005762	tripartite motif-containing 28 (TRIM28)	14.57	31.68	2.2	0.001
NM_020196	XPA binding protein 2 (XAB2)	0.55	1.17	2.1	0.032
NM_015117	zinc finger CCCH type domain containing 3 (ZC3HDC3)	1.65	3.26	2.0	0.001
M94046	zinc finger protein (MAZ)	9.54	25.58	2.7	0.0001
NM_016202	zinc finger protein 580 (ZNF580)	13.05	29.31	2.2	0.007
NM_016535	zinc finger protein 581 (ZNF581)	2.26	6.33	2.8	0.040
	Cell cycle/Cell fate				
NM_006716	activator of S phase kinase (ASK)	6.70	3.46	0.5	0.015
NM_004765	B-cell CLL/lymphoma 7C (BCL7C)	3.00	8.64	2.9	0.026
NM_152434	CWF19-like 2, cell cycle control (CWF19L2)	3.38	1.73	0.5	0.003
NM_017885	host cell factor C1 regulator 1 (XPO1 dependant) (HCFC1R1)	32.14	68.30	2.1	0.022
	Ubiquitin system				
NM_012308	F-box and leucine-rich repeat protein 11 (FBXL11)	7.39	18.42	2.5	0.009
NM_023112	OTU domain, ubiquitin aldehyde binding 2 (OTUB2)	8.11	17.29	2.1	0.049
NM_194457	ubiquitin-conjugating enzyme E2, J2 (UBE2J2)	0.46	1.15	2.5	0.008
	Chaperonins				
NM_016594	FK506 binding protein 11, 19 kDa (FKBP11)	6.16	13.79	2.2	0.003
NM_144617	heat shock protein, alpha-crystallin-related, B6 (HSPB6)	0.24	1.38	5.8	0.008
NM_006431	chaperonin containing TCP1, subunit 2 (beta) (CCT2)	46.14	20.88	0.5	0.016

Menopause. Author manuscript; available in PMC 2015 February 01.

Genbank ACCN#	Gene Name	PreTrt	PostTrt	Fold	p val
	Signal transduction				
	Extracellular matrix				
NM_000088	collagen, type I, alpha 1 (COL1A1)	50.02	160.60	3.2	0.0001
NM_001849	collagen, type VI, alpha 2 (COL6A2)[a]	0.82	2.27	2.8	0.026
NM_199235	collectin sub-family member 11 (COLEC11)	0.89	4.04	4.5	0.007
NM_006691	extracellular link domain containing 1 (XLKD1)	0.72	1.53	2.1	0.001
NM_022664	extracellular matrix protein 1 (ECM1)	0.52	1.19	2.3	0.037
NM_001998	fibulin 2 (FBLN2)	3.88	11.19	2.9	0.002
NM_012445	spondin 2, extracellular matrix protein (SPON2)	1.11	4.12	3.7	0.025
	Proteases and their regulators				
NM_139159	dipeptidylpeptidase 9 (DPP9)	1.31	2.89	2.2	0.001
NM_013379	dipeptidylpeptidase 7 (DPP7)[a]	9.28	23.21	2.5	0.014
BC040958	napsin B aspartic peptidase pseudogene	0.35	1.15	3.3	0.011
	Catalytic/Metabolism				
NM_005787	asparagine-linked glycosylation 3 homolog (ALG3)	1.01	2.18	2.2	0.002
NM_018641	carbohydrate (chondroitin 4) sulfotransferase 12 (CHST12)	2.65	6.75	2.5	0.006
NM_016018	CGI-72 protein (CGI-72)	15.51	7.89	0.5	0.001
NM_001785	cytidine deaminase (CDA)	1.22	3.22	2.6	0.002
NM_014080	dual oxidase 2 (DUOX2)	6.96	16.88	2.4	0.002
NM_000847	glutathione S-transferase A3 (GSTA3)	0.73	2.87	3.9	0.027
AK057656	similar to mitochondrial processing peptidase beta subunit	14.62	7.24	0.5	0.0001
NM_147156	transmembrane protein 23 (TMEM23)	24.98	12.82	0.5	0.011
NM_033452	tripartite motif-containing 47 (TRIM47)	1.56	4.99	3.2	0.001
NM_003383	very low density lipoprotein receptor (VLDLR)	11.89	5.46	0.5	0.013
	Adhesion				
NM_016174	cerebral endothelial cell adhesion molecule 1 (CEECAM1)	6.36	13.56	2.1	0.004
NM_005529	heparan sulfate proteoglycan 2 (perlecan) (HSPG2)	0.83	5.73	6.9	0.015
NM_021181	SLAM family member 7 (SLAMF7)	3.27	7.98	2.4	0.0001
	Nucleic acid regulation				

Genbank ACCN#	Gene Name	PreTrt	PostTrt	Fold	p val
	Signal transduction				
NM_016652	Crn, crooked neck-like 1 (CRNKL1)	10.35	5.31	0.5	0.007
NM_006773	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18 (DDX18)	12.13	6.12	0.5	0.002
NM_004396	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5 (DDX5)	5.76	1.52	0.3	0.001
NM_002695	polymerase (RNA) II (DNA directed) polypeptide E, 25kDa (POLR2E)	0.57	1.18	2.1	0.001
NM_012232	polymerase I and transcript release factor (PTRF)	3.57	7.11	2.0	0.009
NM_015450	protection of telomeres 1 (POT1)	13.08	6.53	0.5	0.0001
	Ribosomal				
NM_032478	mitochondrial ribosomal protein L38 (MRPL38)	4.00	11.13	2.8	0.002
NM_001011	ribosomal protein S7 (RPS7)	78.30	38.04	0.5	0.040
	Vesicle transport				
NM_016103	SAR1a gene homolog 2 (SARA2)	12.93	6.55	0.5	0.002
NM_138567	synaptotagmin VIII (SYT8)	0.34	1.07	3.1	0.008
NM_013333	epsin 1 (EPN1)	7.82	15.67	2.0	0.001
	Channels/transporters				
NM_004317	arsA arsenite transporter, ATP-binding, homolog 1 (ASNA1)	0.50	1.08	2.2	0.022
NM_021902	FXYD domain containing ion transport regulator 1 (phospholemman) (FXYD1)	24.91	69.35	2.8	0.029
NM_017458	major vault protein (MVP)	1.17	2.56	2.2	0.033
NM_199037	sodium channel, voltage-gated, type I, beta (SCN1B)	2.18	6.33	2.9	0.041
	Unknown				
NM_152285	arrestin domain containing 1 (ARRDC1)[a]	0.74	1.83	2.5	0.007
NM_024083	alveolar soft part sarcoma chromosome region, candidate 1 (ASPSCR1)	2.66	5.51	2.1	0.006

[a] Differentially expressed both in response to treatment with estradiol and in the presence of atherosclerosis as reported previously.[11]

Table 2

Body weight and plasma lipids in control (n=4) and estradiol-treated (n=4) groups at baseline prior to treatment and across 8 months of treatment.

	Baseline		Treatment		ANCOVA
	Control	**Estradiol**	**Control**	**Estradiol**	**p**
BW	3.49±0.39	2.93±0.0.39	3.45±0.43	3.11±0.38	0.83
TPC	338.75±44	376.00±44	263 ± 43	288 ± 62	0.98
TG	46.3 ± 4.8	37.3 ± 4.9	38.8 ± 2.5	51.8 ± 7.7	0.03
HDLC	55.0±10.3	34.50±10.3	45.8 ± 8.0	26.0±6.3	0.14
LDLC+VLDLC	283.8 ± 49	341.50±49	217 ± 51	262 ± 68	0.99
TPC/HDLC	9.23 ± 3.6	13.7 ± 3.7	7.41 ± 3.2	15.8 ± 6.5	0.36

Data during the treatment phase were mean values over the 8 month treatment period. Effects of treatment were assessed using baseline values as covariates. Abbreviations used: Body weight (BW), total plasma cholesterol (TPC, mmol/L), triglycerides (TG, mmol/L), high density lipoprotein cholesterol (HDLC, mmol/L), low density lipoprotein plus very low density lipoprotein cholesterol (LDLC + VLDLC, mmol/L). Statistical significance is denoted by *p*.