Published in final edited form as:

Atherosclerosis. 2008 January ; 196(1): 106–113. doi:10.1016/j.atherosclerosis.2007.02.007.

Effects of Dietary Soy Protein on Iliac and Carotid Artery Atherosclerosis and Gene Expression in Male Monkeys

Sara E. Walker, M.S., Michael R. Adams, D.V.M., Adrian A. Franke, Ph.D.^{*}, and Thomas C. Register, Ph.D.

Comparative Medicine Clinical Research Center (SEW, TCR, MRA), Wake Forest University School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157-1040

Abstract

Male cynomolgus macaques (n=91) consumed an isoflavone (IF)-free, atherogenic control diet containing casein/lactalbumin for five months, then were randomized to three groups: Control (n=30) continued on the control diet; Low IF (n=30) received a mixture of unmodified and IF-depleted soy protein isolate (SPI) (0.94 mg IF/g protein, approximating a human intake of 75 mg/day); and High IF (n=31) received unmodified SPI (1.88 mg IF/g protein, approximating a human intake of 150mg/day) for 31 months. Iliac and carotid artery atherosclerosis, and arterial and hepatic mRNA transcripts related to inflammation and estrogen receptors (ER) were measured. Trend analysis identified a significant inverse relationship between dietary IF content and plaque area in the iliac (p<0.05) but not carotid arteries (p>0.13). No significant effect of diet on inflammatory gene or estrogen receptor expression was observed. Plaque area was positively correlated with the mRNA transcript levels for arterial MCP-1, ICAM-1, and the macrophage marker CD68 (all r>0.25, p<0.03), and negatively correlated with ER α and ER β (all r<-0.23, p<0.03). Coronary artery plaque area appeared to be more closely associated with gene expression patterns of the iliac arteries than the carotid arteries. The data suggests benefits of dietary soy on atherosclerotic plaque development in males may be mediated through inflammation-independent pathways. The negative associations of arterial ER α expression with atherosclerosis lend support to a mechanistic role for estrogen receptors in atherosclerosis susceptibility which merits further study.

Keywords

Atherosclerosis; inflammation; macrophage; plaque; estrogen receptor; cynomolgus macaques

Introduction

Dietary soy has long been thought to have positive health benefits with respect to atherosclerosis-related disease (1–4). However, the roles of the individual soy components, as well as the mechanisms underlying the beneficial effects of soy are poorly understood. Soy protein isolate (SPI) is commonly used in experimental studies addressing possible cardiovascular benefits of dietary soy and is a basic component of many health foods and

CORRESPONDING AUTHOR/REQUEST FOR REPRINTS: Thomas C. Register, Ph.D., Comparative Medicine Clinical Research Center, Wake Forest University School of Medicine, Medical Center Boulevard, Winston Salem, NC 27157-1040 Tele: (336) 716-1557, FAX: (336) 716-1515, e-mail: register@wfubmc.edu.

^{*}The Cancer Research Center of Hawaii, Honolulu, HI 96813

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dietary supplements available to the general public. The major components of soy protein isolate (SPI) most often associated with the suggested cardiovascular benefits of soy are the protein/peptide fractions and the isoflavones (IF). IF are estrogen-like compounds found in soy beans, as well as many legumes and grains (5). The most abundant IF in soy products are daidzein, genistein, and glycitein. In addition, IF metabolites, such as equol, may contribute to the biological activity of dietary IF (5). IF are structurally similar to estradiol and known to interact with estrogen receptors, imparting estrogen agonist/antagonist activities *in vitro* and *in vivo* (6).

Studies with nonhuman primate models have demonstrated inhibitory effects of dietary SPI on atherosclerosis in both male (juvenile and adult) and female (adult) subjects (2,3,7). Sex as well as the timing of intervention may be important issues, as age and or amount of atherosclerosis at initiation may impact outcomes. In both sexes, SPI treatment has been associated with favorable changes in plasma lipid profiles, increasing plasma high density lipoprotein (HDL) cholesterol (HDLc) and decreasing non-HDLc (VLDLc + LDLc) levels. These changes in plasma lipids account for approximately 50% of the decrease seen in atherosclerotic plaque area in these studies (2,7). Our data in post-menopausal female monkeys suggest that dietary soy IF may have anti-inflammatory properties (8), perhaps at the level of the artery wall, although the exact site of such an activity is unclear.

Recently we found that coronary artery atherosclerosis (CAA) in adult male monkeys was reduced by SPI (2). The objective of the present study was to compare and contrast the effects of dietary SPI on atherosclerosis and inflammation in the coronary, iliac and carotid arteries of male cynomolgus macaques. Inflammation was assessed in vascular sites and the liver by measurement of the expression of genes implicated as mediators of inflammation and atherogenesis. We also assessed expression of estrogen receptors as potential mediators of IF effects. We hypothesized that consumption of IF-containing SPI would have anti-inflammatory effects in arteries and/or the liver which would be accompanied by decreased atherosclerotic plaque area.

Materials and Methods

Animals and Diets

This study is an ancillary project to a large trial in cynomolgus males of diet effects on atherosclerosis and behavior, details of which have been previously described (2,9). Male cynomolgus macaques (*Macaca fascicularis*, n=91) were imported as adults from Indonesia (Institute Pertanian Bogor). Animals were fed an isoflavone-free atherogenic induction diet which contained casein lactalbumin (C/L) as the primary protein component. After five months, animals were then placed into three dietary treatment groups using a stratified randomization scheme designed to match the treatment groups for total plasma cholesterol (TPC) and HDLc concentrations. Control animals (n=30) continued to receive the baseline isoflavone-free atherogenic diet with C/L as the major protein source; the low IF group (n=30) was fed a diet containing a mixture of unmodified soy protein isolate (SPI) and IF depleted SPI (0.94 mg total IF/g protein); and the high IF group (n=31) was fed a diet containing unmodified SPI containing 1.88 mg total IF/g protein. The total treatment period was 31 months. Diets were formulated on a caloric basis to contain IF doses corresponding to a human daily consumption of 75 mg (Low IF) or 150 mg (High IF) of IF (as aglycones) per day. Diets were identical in caloric content of protein, fat, and carbohydrate and were calculated to provide 150 cal/kg of body weight daily. All procedures were conducted in compliance with state and federal laws, standards of the U.S. DHHS, and regulations and guidelines established by the Wake Forest University Animal Care and Use Committee.

Necropsy and Atherosclerosis Assessment

At the end of the 31 month treatment period, monkeys were deeply sedated with pentobarbital (30 mg/kg iv.) and euthanized. At necropsy, the liver was removed, sectioned, and frozen in liquid nitrogen. The iliac and carotid arteries were also removed, cleaned of adventitia, opened longitudinally, and sectioned. For the iliac artery, a segment immediately distal to the iliac bifurcation was preserved for histological examination, and an adjacent section was frozen in liquid nitrogen and stored at -80°C for molecular analyses. Carotid arteries were processed in a similar manner, with the section immediately proximal to the aortic arch being preserved for histology, and the adjacent section being frozen in liquid nitrogen and stored at -80°C for molecular analyses. Tissue sections reserved for histological evaluation were placed immediately in fresh 4% paraformaldehyde for 24 hours. Perfusion fixation of the heart and coronary arteries has been previously described (2). Sections were then moved into 70% ethanol, dehydrated, and embedded in paraffin blocks using standard embedding procedures. Tissue blocks were cut into 5 μm thick sections, and stained with Verhoeff and Van Gieson. Plaque area (mm^2) of the left iliac and carotid artery sections were determined by computer assisted histomorphometry using Image Pro Plus software (Media Cybernetics, Inc. Silver Springs, MD; Figure 1). Iliac artery sections were specifically selected as surrogates for the coronary arteries since the coronary artery was perfusion fixed and not optimal for gene expression studies, and atherosclerotic plaque area of the iliac arteries has been shown to be highly correlated with coronary artery atherosclerosis (3).

RNA Isolation & Quantification

RNA was isolated from tissues using the Tri-Reagent protocol as previously described (10, 11). Samples were assessed for total RNA concentration by spectrophotometric analysis at 260 nm, quality was assessed using the A260/A280 ratio and denaturing agarose gel electrophoresis. Aliquots of RNA were reverse transcribed to generate a cDNA archive using a High Capacity cDNA archive kit (Applied Biosystems). Quantitative real time RT-PCR was performed on the ABI Prism 7000 using cynomolgus macaque or human specific Taqman FAM-MGB probe assays (Applied Biosystems, sequences available on request). Individual PCR reactions were carried out using cDNA generated from 40 ng total RNA (arteries) or 80 ng of total RNA (liver). All qRT-PCR data was normalized to the geometric mean of the endogenous constitutively expressed control genes GAPDH, β -actin, and ribosomal protein, large P0 (RPLP, also known as P0, L10E, RPP0, PRLP0) (12). Relative levels of gene expression for each experimental endpoint were calculated against the geometric mean of the endogenous controls using the $2^{-\Delta\text{CT}}$ procedure (13).

Plasma Lipids

Total plasma cholesterol (TPC), HDLc, and plasma triglycerides (TG) were determined at the end of the pre-treatment phase and at the end of the 31 month treatment period in the Comparative Medicine Clinical Research Center Clinical Chemistry and Endocrinology Laboratory, and plasma lipoprotein distributions were determined at baseline and at frequent intervals during the treatment phase as previously described (2).

Serum Hormone Analyses

Estradiol concentrations were determined in serum samples obtained at the end of the 5 month pre-treatment period and the 31 month treatment period. Assays were performed in the Comparative Medicine Clinical Research Center Comparative Endocrinology Laboratory using a modification of a previously described method (14). Serum (0.5 ml) was extracted by addition of ethyl ether (4 ml) followed by inversion for 5 min. Phases were allowed to equilibrate, the aqueous layer was then frozen in a dry ice/isopropanol bath and the organic phase was decanted. Organic extracts were dried and reconstituted with the zero standard serum

from the radioimmunoassay kit (DSL 4800, ultrasensitive estradiol, Diagnostics Systems Laboratories, Webster, TX, USA). Serum testosterone (free and total) and 3 α -androstanediol glucuronide were determined at the end of the 5-month pre-treatment period and after 15-months treatment. Serum total and free testosterone were determined on unextracted samples using solid phase Coat-a-Count radioimmunoassays (Diagnostics Products Corporation, Los Angeles, CA, USA). The androgen metabolite 3 α -androstanediol glucuronide was measured using a coated tube RIA from Diagnostics Systems Laboratories (Webster, TX). Coefficients of variation for the individual assays were as follows: estradiol, 7.9%; total testosterone, 9.6%, free testosterone, 6.8%, and 3 α -androstanediol glucuronide 9.5%.

Serum Isoflavone Analyses

Serum isoflavone analysis was performed using liquid chromatography photo-diode array electrospray mass spectrometry (LC/PDA/ESI-MS). This procedure was slightly modified from previously established protocols to include equol in the isoflavonoid panel (15).

Statistical Analysis

All data were assessed for normality. For data which could not be normalized by transformation, main effects of treatment were determined by non-parametric analyses (Kruskal-Wallis test) to determine significance using the SAS (Cary, NC) statistical software package. Main effects of diet were calculated for plaque area, gene expression, serum steroid hormone, and serum isoflavone data. Trend analysis was also used to determine relationships between plaque area and gene expression measures and diet. Spearman rank-order correlations between molecular endpoints, plaque area, and pre and post-treatment cholesterol, steroid hormones, and post-treatment isoflavone levels were also determined. Significance was defined as p value ≤ 0.05 for the trend analysis of plaque area, which represented a single test within a tissue site, and for the correlation analyses, which were largely exploratory in nature and to be used for hypothesis generation. For the tests for main effects of diet on gene expression, a significance level of 0.01 was used due to the multiple tests ($n=5-6$) for each tissue.

Results

Diet and Atherosclerosis Risk Factors

There were no significant effects of diet on body weight, weight gain, or steroid hormone levels (data not shown). Dietary effects on plasma lipoprotein concentrations described previously as part of a companion report (2) are presented in brief in Supplementary Table 1. Plasma LDL-c was significantly decreased in the Low IF (5.0 mg/dL) and High IF (5.2 mg/dL) treatment groups compared to controls (6.3 mg/dL). Plasma HDL-c was significantly greater in the Low IF (1.5 mg/dL) and High IF (1.3 mg/dL) groups when compared to controls (1.1 mg/dL). As expected, serum isoflavone levels were significantly different between all treatment groups, with control animals having the lowest plasma concentrations, and High IF having the highest (2)

Iliac and Carotid Artery Atherosclerosis

Iliac artery plaque area decreased in a dose-dependent manner with the dietary IF content (trend analysis, $p<0.05$), but this effect was not seen in the carotid artery (trend analysis, $p=0.13$) (Figure 2). Coronary artery atherosclerosis (CAA) (2) was significantly correlated with both iliac ($r=0.58$, $p<0.0001$) and carotid artery atherosclerosis ($r=0.55$, $p<0.0001$). Overall, the iliac arteries had larger atherosclerotic plaque cross-sectional areas (1.12 ± 0.06 mm²) than the coronary (0.51 ± 0.06 mm²) and carotid (0.61 ± 0.06 mm²) arteries ($p<0.01$ for both). Arterial plaque area in all three arteries were highly correlated with post-treatment TPC, non-HDLc,

and TPC/HDL ratio, and negatively correlated with post-treatment HDL-c (Table 1). Iliac artery plaque area was positively correlated with circulating pre-treatment androstenediol glucuronide levels ($r=0.21$, $p<0.05$).

Atherosclerosis and Gene Expression

Arterial and hepatic gene expression (relative to endogenous control genes GAPDH, β -actin, and RPLP) were not affected by dietary treatments (Table 2). Within each artery and across arterial sites, plaque area was positively associated with inflammation-associated gene expression in that artery but not in the liver (Table 3). Likewise, hepatic CRP expression was not related to atherosclerotic plaque area in any of the arteries examined. Iliac plaque area was negatively correlated with both ER α and ER β gene expression, while carotid artery plaque area was negatively correlated with ER α only. Across arterial beds, CAA was negatively correlated with ER α gene expression in both the iliac and carotid arteries. Hepatic ER gene expression was not correlated with plaque area in any of the arteries examined (Table 3).

Transcript levels (relative to endogenous control genes GAPDH, β -actin, and RPLP) significantly differed between artery and liver, as well as between arterial beds (Supplemental Materials Table 2). Vascular expression of MCP-1 was higher than hepatic expression ($p<0.001$, both arteries), while ICAM-1 was found to be higher in the iliac artery than in the liver ($p<0.007$). In contrast, hepatic expression of VCAM-1, ER α , and ER β was higher than vascular expression ($p<0.001$ for all). Vascular transcript levels did not differ between the carotid and iliac arteries, with the exception of ICAM-1 and CD68 which were more highly expressed in the iliac artery than the carotid ($p<0.001$; Supplemental Materials Table 2).

Gene Expression and Plasma Measures

There were no significant effects of treatment on circulating steroid hormone levels (data not shown). Pre-treatment levels of the androgen metabolite androstenediol glucuronide were positively associated with iliac MCP-1 gene expression ($r=0.25$, $p<0.02$). Significant relationships were also observed between gene expression outcomes and plasma lipid concentrations assessed during the treatment period (Supplemental Table 3). For example, iliac expression levels of MCP-1, ICAM-1, and CD68 were positively correlated with plasma TPC, non-HDLc, and TPC/HDLc ratio, and negatively associated with HDLc assessed during the treatment phase. Iliac ER α and ER β expression were negatively correlated with TPC, non-HDLc, and TPC/HDLc ratio, while treatment levels of HDLc were positively correlated with ER β alone. Carotid artery gene expression measures followed a similar pattern of associations, although carotid artery ER β expression was not correlated with TPC concentration and was correlated with serum estradiol ($r=0.25$, $p<0.02$). Hepatic transcripts related to inflammation and ER expression were not correlated with post-treatment plasma lipid measures.

Discussion

The purpose of this study was to determine the effects of dietary SPI on atherosclerosis, on arterial and hepatic expression of known inflammatory mediators implicated in atherogenesis, and on expression of estrogen receptors in adult male monkeys. We found that dietary SPI had an inhibitory effect on coronary and iliac artery atherosclerosis, but no statistically significant effect on carotid artery atherosclerosis or on inflammatory gene expression in arteries or liver. The results indicate that soy and/or soy IF did not have sustained inhibitory effects on inflammatory genes, in contrast to previous findings on circulating sVCAM-1 in ovariectomized female non-human primates (8) and on hepatic inflammatory gene expression in ovariectomized mice (16). It is possible the observed differences may relate to sex-specific effects of isoflavones in ovariectomized female models, which have low circulating levels of endogenous hormones.

Our assessments were performed on male arteries collected after 31 months of continuous atherogenic diet consumption and atherosclerotic lesions were relatively advanced, some lesions having large areas that were necrotic and relatively acellular. As suggested by previous studies of Clarkson, et al (3), more advanced lesions may be less responsive to intervention than early lesions. Since this is a cross-sectional examination of gene expression after several years of treatment, we cannot rule out the possibility that anti-inflammatory effects may have been present earlier which were no longer apparent due to the more advanced stage of development of the atherosclerotic lesions. On the other hand, it is possible, and perhaps likely, that the anti-atherogenic effects of SPI in this study are mediated through inflammation-independent pathways, such as by inhibition of vascular smooth muscle cell migration and proliferation and/or plaque matrix accumulation.

While findings relative to effects of SPI on the expression of inflammatory genes implicated in atherosclerosis were largely negative, there were new findings relative to gene expression differences across tissues, their relationship with atherosclerosis and plasma lipid levels, and in particular relationships between estrogen receptor expression and atherosclerosis. We found that iliac artery atherosclerosis was inversely related to IF dose, while in the coronary arteries both Low IF and High IF diets produced similar reductions in plaque size compared to control (2). Carotid artery atherosclerosis was not significantly reduced by diet or related to IF dose, although the pattern was similar to that of the iliac arteries. These differential responses in individual vascular beds could be indicative of different IF dose thresholds required for atherosclerosis inhibition, with the coronary being most sensitive and the peripheral arteries being more resistant/less responsive to the lower dose of IF.

Since the fixative used for histological assessment of the coronaries rendered them imperfect for gene expression studies, iliac arteries were used as a surrogate for the coronary arteries. The current study confirmed the results of previous studies in showing that iliac artery plaque area was highly correlated with CAA (3,8), and iliac artery gene expression was more highly correlated with CAA than was carotid artery gene expression. These findings parallel studies in human populations reporting that coronary artery calcification was more highly correlated with calcification in the iliac arteries than in the carotid arteries (17). Iliac artery atherosclerosis has been associated with future coronary events in people (18). Together, these findings support the use of iliac arteries as surrogates for coronary arteries.

Carotid arteries differed from iliac arteries, not only in atherosclerotic plaque area, but also in gene expression. While carotid arteries generally develop smaller plaques than do iliac arteries (this report, 3, 17), it is unknown if this is due to delayed initiation of lesion development, to slowed progression, or both, relative to iliac artery plaques. Carotid arteries had significantly lower expression of both MCP-1 and ICAM-1 than did iliac arteries, which may have resulted in less monocyte adhesion and recruitment and smaller plaque size relative to the iliac arteries. This assumption is supported by the finding that iliac artery CD68 expression was significantly higher than carotid artery CD68 expression, suggesting a higher macrophage content within the iliac artery compared with the carotid.

Hepatic gene expression of inflammatory markers was found to be unrelated to atherosclerotic plaque area in this study. Liver is the major producer of CRP, and circulating CRP is an independent risk factor for cardiac events (19). CRP has also been shown to be produced in atherosclerotic lesions (20), and to induce adhesion molecule expression in endothelial cells (21), perhaps playing a direct role in promoting the inflammatory component of atherosclerosis. However in the Dallas Heart Study circulating CRP levels were not directly associated with atherosclerosis extent (22), similar to our own findings in female cynomolgus monkeys (8). The current study extends these observations to the liver, as hepatic CRP transcript levels were not correlated with any atherosclerotic plaque measures.

Adhesion molecules, such as VCAM-1, promote monocyte adhesion to the arterial endothelium and have been associated with increased atherosclerosis. For example, transgenic LDL-R knockout mice defective in the expression of normal VCAM-1 had reduced susceptibility to atherogenesis (23). Dietary SPI/IF significantly decreased serum VCAM-1 levels in a long term study of surgically post-menopausal female macaques (8), and in post-menopausal women in a genotype dependent manner (24), but not in two short term dietary studies of 6 weeks (25) and 20 weeks (26) duration. We hypothesized that reduced arterial VCAM-1 might provide a mechanistic basis for anti-atherogenic effects of IF. However, we observed no change in VCAM-1 mRNA expression in any of the tissues examined, and no relationship between arterial VCAM-1 mRNA expression and plaque size in this study. These findings suggest that any relationships between soy effects on arterial VCAM-1 expression and atherogenesis may be temporal in nature, related more to VCAM-1 protein than mRNA, and/or are undetectable at the mRNA level in the intima-media preparations in the present study. It is also possible that most of the atheroprotective effects of dietary soy in males relate to beneficial influences on plasma lipids and lipoproteins, and/or are dependent on other mechanisms, perhaps by inhibition of SMC proliferation or extracellular matrix accumulation.

While there was no effect of SPI on ER expression, ER α was negatively correlated with plaque area in the iliac and carotid arteries. Previous studies suggest that the anti-atherosclerotic effects of soy are in part mediated through ER α -dependent processes (1). It is unclear if the negative correlation between arterial plaque area and ER gene expression is due to an ER-dependent effect on plaque development and progression, or if the decreased ER expression is a consequence of increased plaque size and complexity perhaps related to altered plaque cellular composition or biology.

Some studies suggest that atheroprotective effects of SPI may be mediated in part through a direct influence on the artery (1,2,7,27). We observed no effect of SPI on arterial expression of several NF- κ B dependent inflammation- and atherosclerosis-related genes in the current study. The methods used for these studies preclude us from assessing longitudinal effects on the artery, or of measuring any effect on gene expression which may have been present during earlier periods of lesion development. The studies are limited by the fact that the inflammation and ER specific gene expression assays were performed on homogenates of intimal-medial preparations of arteries, and we have no data on expression levels by individual cell types. In addition, since we focused on expression of mRNA only, we have no information on possible effects of SPI on the steady state levels of the protein products of these genes, particularly with respect to expression of adhesion molecules along the endothelium, a potential target of interventions. Nevertheless, the findings suggest minimal anti-inflammatory effects of dietary soy and soy IF in males.

It is important to note that the study design used in this experiment does not allow one to dissect the effects of the protein components of soy from those of the isoflavones and other constituents. Though dietary levels of IF comparable to that attained in people using dietary supplements were used here, the range (75 and 150 mg/day) is relatively narrow. In addition, the effects of soy isoflavones on atherogenesis in males may be quite distinct from those in surgically postmenopausal females. Circulating concentrations of the sex hormones estradiol and testosterone are higher in both young and adult males than those found in ovariectomized and post-menopausal females, and the relative influence of isoflavones in such a hormonal environment may be blunted or altered. Estrogens are known to have potent effects in males, as evidenced from studies of men with mutations in ER α or the aromatase enzyme in which premature osteopenia/osteoporosis and vascular calcification, as well as abnormal vasodilatory responses have been observed (28–30). More recently, higher serum estradiol levels at baseline were found to be associated with lower risk of CHD events in older men in the Framingham Heart Study (31). The variations in the levels of ER expression in different arterial beds and

the relationship between ER expression and atherosclerotic plaque area observed in the current studies merit further exploration, as the level of ER expression within an artery could play a role in atheroprotection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This project was supported in part by NIH grants HL45666 (MRA), and AG18170 (TCR). The authors gratefully acknowledge The Solae Company for their generous contributions of soy products; and thank Gerald Perry, Debbie Golden, Megan Phillips, Dewayne Cairnes, Melissa Ayers, and Cheryl Wilmoth for their technical contributions. This manuscript includes 2 figures, 3 tables, and 3 supplemental tables.

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Figure 1.

Composite figure of images of representative VVG stained sections of coronary, iliac, and carotid arteries from each of the treatment groups. Sections of arteries representing the median plaque area measurement for each treatment group are shown. All sections are shown at 40x magnification with the luminal side of the artery toward the top of the page.

Figure 2.

Relative mean of coronary, iliac, and carotid artery plaque areas expressed as percent of control group plaque area. Coronary 100% = 0.65 mm², Iliac = 1.31 mm², and Carotid = 0.73 mm². Iliac artery plaque area decreased with increasing IF concentration (p for trend <0.05), there was no effect on carotid artery (p for trend=0.13). Coronary artery data is reproduced with permission from the Journal of Nutrition (2005; 135:2855). * differs from control group in the respective artery p<0.05.

Table 1
Spearman rank order correlations of plaque area measures in individual arteries with post-treatment plasma lipids.

	Iliac	Carotid	Coronary	P-value
	Artery (r)	Artery (r)	Artery (r)	P-value
TPC	0.46*	0.52*	0.56*	<0.0001
TG	0.05	0.01	-0.17	0.10
HDL	-0.35*	-0.27*	-0.51*	<0.0001
LDL+VLDL	0.49*	0.53*	0.60*	<0.0001
TPC/HDL	0.50*	0.48*	0.66*	<0.0001

* represents significant correlations p<0.05.

Table 2

Iliac artery, carotid artery, and hepatic gene expression measures in treatment groups relative to expression levels in the control group. Measures were first normalized to the geometric mean of GAPDH, B-actin, and RPLP mRNA levels. IF treatment had no effect on arterial or hepatic inflammatory or ER gene expression.

		Control $\pm$ s.e.	Low IF $\pm$ s.e.	High IF $\pm$ s.e.	P-value
Iliac n=91	MCP-1	100 $\pm$ 14.9	89.4 $\pm$ 15.2	83.4 $\pm$ 14.7	0.64
	VCAM-1	100 $\pm$ 11.1	92.3 $\pm$ 11.3	98.2 $\pm$ 11.0	0.85
	ICAM-1	100 $\pm$ 11.3	76.2 $\pm$ 11.8	92.7 $\pm$ 11.3	0.36
	CD68	100 $\pm$ 17.7	71.5 $\pm$ 17.9	75.1 $\pm$ 17.5	0.95
	ER α	100 $\pm$ 11.5	125 $\pm$ 11.7	113 $\pm$ 11.3	0.43
	ER β	100 $\pm$ 14.7	127 $\pm$ 15.3	133 $\pm$ 14.7	0.25
Carotid n=88	MCP-1	100 $\pm$ 38.9	109 $\pm$ 38.9	164 $\pm$ 38.1	0.65
	VCAM-1	100 $\pm$ 45.7	74.9 $\pm$ 44.9	23.3 $\pm$ 44.0	0.17
	ICAM-1	100 $\pm$ 13.6	106 $\pm$ 13.9	102 $\pm$ 12.9	1.00
	CD68	100 $\pm$ 17.9	89.9 $\pm$ 17.9	99.1 $\pm$ 17.2	0.94
	ER α	100 $\pm$ 14.4	110 $\pm$ 14.4	110 $\pm$ 13.4	0.75
	ER β	100 $\pm$ 18.6	125 $\pm$ 18.2	129 $\pm$ 17.9	0.49
Liver n=88	MCP-1	100 $\pm$ 13.3	90.5 $\pm$ 13.3	76.2 $\pm$ 12.9	0.28
	VCAM-1	100 $\pm$ 9.40	93.2 $\pm$ 9.40	79.5 $\pm$ 9.40	0.34
	ICAM-1	100 $\pm$ 10.0	112 $\pm$ 10.0	90.0 $\pm$ 9.38	0.22
	CRP	100 $\pm$ 27.9	95.1 $\pm$ 27.9	148 $\pm$ 27.1	0.64
	ER α	100 $\pm$ 10.1	117 $\pm$ 10.1	100 $\pm$ 9.75	0.45
	ER β	100 $\pm$ 16.7	93.3 $\pm$ 16.7	80.0 $\pm$ 16.0	0.32

Table 3

Spearman rank order correlations of gene expression measures in the iliac artery, the carotid artery, and the liver with atherosclerotic plaque sizes in the coronary, iliac, and carotid arteries. In general, inflammatory gene expression in the arteries was positively correlated with atherosclerosis in multiple beds, while hepatic inflammatory gene expression was not. Arterial estrogen receptor expression was negatively correlated with atherosclerosis plaque areas.

Gene Expression	Coronary Artery (r)	P-value	Iliac Artery (r)	P-value	Carotid Artery (r)	P-value
Iliac n=91						
MCP-1	0.46*	<0.0001	0.52*	<0.0001	0.54*	<0.0001
VCAM-1	0.18	0.10	0.14	0.18	0.02	0.86
ICAM-1	0.32*	0.01	0.50*	<0.0001	0.38*	0.001
CD68	0.43*	<0.0001	0.60*	<0.0001	0.44*	<0.001
ER α	-0.39*	0.001	-0.23*	0.03	-0.23*	0.03
ER β	-0.45*	<0.0001	-0.33*	0.001	-0.36*	0.001
Carotid n=88						
MCP-1	0.11	0.33	0.12	0.26	0.25*	0.02
VCAM-1	0.04	0.70	0.32*	0.003	0.03	0.82
ICAM-1	0.21*	0.05	0.27*	0.01	0.38*	0.001
CD68	0.42*	<0.0001	0.38*	0.001	0.67*	<0.0001
ER α	-0.30*	0.01	-0.33*	0.003	-0.46*	<0.0001
ER β	-0.23*	0.04	-0.20	0.08	-0.25*	0.02
Liver n=88						
MCP-1	0.05	0.68	0.13	0.24	0.002	0.99
VCAM-1	0.05	0.62	0.09	0.39	0.06	0.61
ICAM-1	0.09	0.40	0.15	0.15	0.12	0.27
CRP	-0.04	0.73	-0.07	0.53	-0.14	0.19
ER α	-0.14	0.18	-0.07	0.54	-0.04	0.75
ER β	0.15	0.15	0.18	0.10	0.03	0.77

* represents significant correlations ($p < 0.05$).