Published in final edited form as:

Reprod Sci. 2010 June ; 17(6): 511–531. doi:10.1177/1933719110367829.

Gender Differences in Cardiovascular Disease: Hormonal and Biochemical Influences

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Abstract

Objective—Atherosclerosis is a complex process characterized by an increase in vascular wall thickness owing to the accumulation of cells and extracellular matrix between the endothelium and the smooth muscle cell wall. There is evidence that females are at lower risk of developing cardiovascular disease (CVD) as compared to males. This has led to an interest in examining the contribution of genetic background and sex hormones to the development of CVD. The objective of this review is to provide an overview of factors, including those related to gender, that influence CVD.

Methods—Evidence analysis from PubMed and individual searches concerning biochemical and endocrine influences and gender differences, which affect the origin and development of CVD.

Results—Although still controversial, evidence suggests that hormones including estradiol and androgens are responsible for subtle cardiovascular changes long before the development of overt atherosclerosis.

Conclusion—Exposure to sex hormones throughout an individual's lifespan modulates many endocrine factors involved in atherosclerosis.

Keywords

Cardiovascular disease; atherosclerosis; hormones; factors; gender

INTRODUCTION

The association between atherosclerosis and cardiovascular health has long been a source of investigation. As early as 1933, an association between dietary changes and female serum cholesterol levels was reported.¹ In 1958, during a conference on “Hormones and Atherosclerosis,” researchers concluded that cholesterol deposition was directly linked to blood cholesterol levels, although other factors, including gender, hormones, diet, lipid levels, and stress, were deemed important as well. In particular, it was reported that ethinyl estradiol reduced plasma lipid levels in a group of men with previous myocardial infarction

(MI), although there was no significant reduction in morbidity or mortality from coronary events as compared to the control group.²

Despite years of research, however, the relationships between cholesterol, atherosclerosis and cardiovascular risk (CVR) remain controversial.³⁻⁸ The popularity of statins and other cholesterol-lowering medications is a testament to the notion that elevated cholesterol is linked to heart disease. The real connection, however, is likely not so straightforward. Half of individuals who have a heart attack do not have high cholesterol levels, and some of the cardioprotective effects of statins seem to be mediated via a reduction in arterial inflammation^{9,10} and effects on vitamin D rather than through a simple lowering of cholesterol.^{11,12} Furthermore, there is not enough information regarding the long-term impact of pharmacologic treatment of cholesterol levels. It is known that statins inhibit cerebral cholesterol synthesis by blocking the growth of new nerve cell synapses; this may account for some of their reported adverse effects such as amnesia, confusion, disorientation, and dementia.^{13,14} Statins also inhibit the production of other vital biochemicals, notably the potentially cardioprotective co-enzyme Q10.¹⁵

Because the connection between hypercholesterolemia and CVD is not a straightforward one, researchers have been actively investigating other potential contributors. The role of sex hormones in cardiovascular disease (CVD) is an emerging field of interest. A small series of men with deficient estrogen action (due to mutations in the genes for estrogen receptor [ER] or aromatase) developed early-onset atherosclerosis, increased visceral adipose tissue, hyperinsulinemia, and the constellation of risk factors known as metabolic syndrome. These same abnormalities have also been reported in men with sufficient estrogen action but low testosterone (T) levels.¹⁶ It has been postulated that the described alterations in sex hormone alterations may cause these cardiovascular abnormalities and that, ultimately, atherosclerotic CVD may be an endocrinological disorder.¹⁶

In the last decade, newer hormones, including leptin, adiponectin, and vitamin D, have also been linked to different phases of vascular dysfunction.¹⁷⁻¹⁹ Despite 50 years of research, however, there is no unifying theory for the pathophysiology of CVD, which might allow for development of effective therapies with fewer side effects than those currently available. We present a review of recent evidence regarding influencing factors, with a focus on gender-specific influences that may be involved in the genesis of CVD.

THE CARDIOVASCULAR GENDER GAP

Epidemiological, clinical, and experimental data have provided evidence that there are gender-specific variations in CVR. From a broad perspective, many medical comorbidities, including endocrine disorders such as hypothyroidism and diabetes and psychiatric disorders such as depression, manifest a gender predilection and are associated with increased CVR.²⁰ For example, women are more likely than men to experience an episode of depression and to be subsequently diagnosed with the metabolic syndrome.²¹ The prevalence of subclinical hypothyroidism increases with age and is higher in women, and several studies have suggested an association between thyroid disease and coronary artery disease.²² Finally, women with diabetes are less likely to be well-controlled (as manifested by glycosylated hemoglobin [HbA1C] levels less than 7%) and are more likely to develop and die from CVD.²³

Apart from these and other comorbidities, the menopausal period in itself appears to be a time of transition to increased CVR.²⁴ The incidence of nondiabetic CVD is lower in premenopausal women than in age-matched men.²⁵ Ovarian hormone insufficiency at the time of the menopause is associated with increased cardiovascular events. Thus, CVD develops, on average, 10 years later in women compared to men.²⁶ These observations have

led to the development of the hypothesis that estrogens protect women against atherosclerotic complications.

At the cellular and biochemical level, gender differences in the regulation of physiological mechanisms are directly influenced by genetic polymorphisms. Some studies have linked CVD with variations in the nuclear hormone family of ER genes, including ER- α gene (ESR1) and ER- β gene (ESR2).²⁷ These receptors function as ligand-dependent transcription factors and predominate in vascular endothelial and smooth muscle cells.²⁸ Estrogen receptor α gene polymorphisms may influence response to peripheral estradiol and, indirectly, the prevalence of different menopause-related conditions.²⁹ Postmenopausal women who carry a particular ESR1 variant (haplotype 1, c.454–397 T allele and c.454–351 A allele) are at increased risk of MI and ischemic heart disease (IHD), independent of known CVR factors.³⁰ This association has not been observed in men.³¹

Estrogen receptor β gene expression predominates in human vascular smooth muscle cells (VSMCs).³² In rat models, expression of ESR2 was induced after vascular injury and its expression is associated with increased coronary artery plaque surface area in both women and men.^{33,34} Some ESR2 polymorphisms are associated with gender-specific increased risk of CVD (particularly MI). For example, women with a particular variant allele (RS127152) had significantly increased risk of CVD and MI, while men with a different rare variant (RS1256049) were at reduced risk. Other variants, however (such as RS127152), confer increased risk of MI upon men only.³⁵ In postmenopausal women, a near-significant association was found between some ESR2 haplotypes and obesity—a related CVD condition.³⁶

Selective stimulation of a second type of ER, the intracellular G protein-coupled ER (GPER), decreases rat blood pressure and dilates human arterial blood vessels.³⁷ In GPER knockout mice, this effect is abolished and visceral obesity is observed. Female GPER knockout mice had impaired glucose tolerance (hyperglycemia), reduced body growth, increased blood pressure, and reduced insulin-like growth factor blood levels.³⁸

In women, endogenous female sex hormones, especially estrogens, are cardioprotective via multiple mechanisms: increased high-density lipoprotein (HDL), decreased low-density lipoprotein (LDL), and release of vasodilators such as nitric oxide (NO) and prostacyclin (PGI₂) from vessel walls, which results in inhibition of vascular constriction and lowering of blood pressure as well as decreased platelet aggregation.³⁹ In a study in which men and women underwent 24-hour ambulatory blood pressure monitoring, men had higher blood pressure than their age-matched, premenopausal female counterparts. However, after menopause, blood pressure increased in women to levels even higher than those observed in men.⁴⁰ In addition, during the menopausal transition, there is an increase in the prevalence of the metabolic syndrome, elevated body weight, dyslipidemia, hyperinsulinemia, and hypertension.^{41,42} Hormone therapy (HT), in most cases, does not significantly reduce blood pressure in postmenopausal women, suggesting that estrogen deficiency may not be the only component involved in postmenopausal hypertension.⁴²

The lower prevalence of CVD in premenopausal women, in comparison to age-matched men, has been explained by differences in body fat distribution, plasma lipoprotein levels, and indices of plasma glucose-insulin homeostasis.^{43,44} Women generally have a higher plasma HDL levels and lower plasma insulin, apolipoprotein B, and triglyceride (TG) levels (which have been associated with abdominal visceral adipose tissue).⁴⁵ Additionally, estrogen seems to contribute to glucose homeostasis via increased glucose transport into the cell, and studies have suggested a positive effect of hormone replacement therapy on HbA1C levels in postmenopausal women.⁴⁶

SEX HORMONES AND LIPOPROTEINS

Although the mechanism by which exogenous estrogen may prevent CVD is most likely multifactorial, during the last decades, increased emphasis has been given to estrogen's effect on the lipid profile and the process by which alterations in the lipid profile lead to CVD. High serum lipid levels (especially LDL) contribute to atherosclerosis.^{47,48} This process seems to be initiated at the level of the intima via macrophage uptake of oxidatively modified LDL, which results in release of the proinflammatory cytokine tumor necrosis factor α (TNF- α) from monocytes and macrophages.⁴⁹ Oxidized LDL induces apoptosis in cultured endothelial cells and promotes inflammation via enhancement of in vitro endothelial cell monocyte adhesion.⁵⁰ In vitro preparations, 17 β estradiol at high local concentrations inhibits LDL oxidation and reduces cholesterol ester formation^{51,52}; T does not have the same effect.⁵²

However, estrogen's LDL-lowering effect accounts only for 25% of the observed reduction in CVR.⁵³ A high fraction (66%) of women included in the Framingham Cohort, who had incident coronary heart disease, did not have either hypercholesterolemia or elevated serum LDL. These women did, however, have elevated TG levels and low HDL levels.⁵³ The combination of elevated LDL and elevated TG levels predicts a heightened risk of CVD, beyond the prediction obtained by elevated LDL alone.⁵⁴ Hypertriglyceridemia also decreases high-density lipoprotein cholesterol (HDL-C) while increasing insulin resistance, glucose intolerance, hypertension, and prothrombotic states with the overall effect of further increasing CVR.⁵⁴ There was also a trend for men to have higher LDL concentrations than women and for women to have higher HDL concentrations than men.⁵⁴ This difference in HDL particle size decreased with age, with a concomitant increase in CVR.⁵⁴ An additional CVD risk factor is elevated apolipoprotein (a) levels that tend to increase after menopause.⁵⁵

Gender differences in endogenous sex hormones and lipoprotein subfractions were confirmed via nuclear magnetic resonance (NMR) spectroscopy.⁵⁶ When VLDL, LDL, and HDL particle size and numbers were measured in men and non-HT-using postmenopausal women included in the Multi-Ethnic Study of Atherosclerosis (MESA) baseline examination, a more atherogenic lipo-protein particle profile was associated with higher endogenous estradiol and lower sex hormone binding globulin (SHBG) levels.⁵⁷

The Women's Health Initiative (WHI) investigated the role of conjugated equine estrogen (CEE), alone or in combination with progestin, in decreasing cardiovascular events in women.⁵⁸ While the trials were discontinued early due to an increased risk of CHD and stroke,⁵⁹ the trials are limited by the choice of an older population (with a mean age of 63 years at the time of initiation of HRT: hormone replacement therapy (or hormone therapy)).⁶⁰ Subsequent subgroup analyses suggest that women who initiate HT in the early postmenopausal period have lower CVR than those who initiate HT more than 20 years after menopause.⁶¹ In addition, an analysis of the WHI trial has found that younger postmenopausal women treated with the estrogen-only regimen had significantly less coronary artery calcification than their counterparts taking placebo.⁶² These data suggest that estrogen may decelerate the early stages of atherosclerosis. Women in the early postmenopausal years undergo fewer thrombotic modifications to the vasculature during HT, because the endothelium is healthier and more resistant to thrombosis.⁶³⁻⁶⁵ Disappointingly, late initiation of HT has not been associated with cardiovascular benefits.^{58,59} Furthermore, when ET has been used in men (eg, as an adjuvant component for prostate cancer chemotherapy), it has been associated with serious adverse vascular effects and increased cardiovascular-related deaths.⁶⁶ The appropriate route of administration is also controversial. Some of the adverse effects of estrogen are related to the "first-pass effect" through the portal circulation, an effect that can be avoided by

transdermal estrogen administration, thus avoiding hepatic enzyme induction.⁶⁷ Additionally, there are data to suggest that transdermal estrogen may be more effective at lowering blood pressure.⁶⁷ However, the hepatic effects of oral estrogens are also responsible for the improved lipoprotein levels seen after initiation of HT. The Kronos Early Estrogen Prevention Study (KEEPS) trial will address many of these issues in younger, recently postmenopausal women, comparing transdermal and oral estrogen in a blended trial.⁶⁸

ESTROGEN'S EFFECTS ON THE VASCULATURE

Estrogen-induced lipoprotein profile changes partly explains estrogen's cardioprotective effect in premenopausal women.⁶⁹ In addition, estrogen and its receptors are involved in multiple other biochemical pathways, including stimulation of angiogenesis, endothelial NO production, and regulation of cytokines and inflammatory markers that may help to explain their antiatherosclerotic effects.⁷⁰⁻⁷² Steroid receptors—and specially ERs—interact with a wide range of coregulatory factors that may change depending on endothelial age and alteration. This physiological cross talk and biological equilibrium may be abruptly interrupted by estrogen deprivation and can ultimately increase an individual's risk of CVD.⁷³⁻⁷⁶

Estrogens exert direct effects on arterial wall smooth muscle to cause vasodilation.^{77,78,87,88} Estrogens produce rapid vasodilatory effects by increasing endothelial nitric oxide synthase (eNOS) activity, an effect that does not require changes in gene expression.⁷⁹ In the long term, estrogen's effects on vasodilatation are mediated, at least partly, by changes in expression of the estrogen-specific receptors ESR1 and ESR2. Estrogen mediates arterial vasodilatation and blood flow through this pathway via stimulation of endothelial PGI₂ synthesis and inhibition of endothelin-1 (ET-1), a substance that has a vasoconstrictive effect on arterial subendothelial smooth muscle cells.^{80,81} Also notably, in vitro studies using porcine coronary arteries contracted with a thromboxane A₂ (TXA₂) analogue showed direct relaxation after addition of increasing concentrations of estradiol and estrone.⁸²

Surgically induced menopause causes an increase in peripheral vascular resistance and blood pressure. The observation that blood pressure often returns to baseline after initiation of HT suggests that ovarian (and primarily estrogenic) insufficiency accelerates development of hypertension.⁸³ The effect of exogenous estrogen on blood pressure may depend on the type, route, and dose of estrogen administered. In normotensive postmenopausal women, transdermal estradiol decreases sympathetic nerve discharge without augmenting arterial baroreflexes, causing a small but significant decrease in ambulatory blood pressure.^{84,85} Sympathetic inhibition is evident only with chronic rather than acute estrogen administration, implying a genomic mechanism of action.⁸⁵⁻⁸⁸

Estradiol also acutely modulates vascular activity of vasoconstrictors such as angiotensin or serotonin, regulating venous endothelin receptor expression and stimulating vasoconstrictor prostanoids. In nonatherosclerotic human coronary arteries, estradiol induces rapid endothelium-independent vasodilatation and enhances bradykinin endothelium-dependent relaxation.^{89,90} As will be further discussed, all these data point out to the fact that nongenomic estradiol pathways are relevant to normal vasodilation.

ANDROGENS AND THE CARDIOVASCULAR SYSTEM

Links between androgens and the cardiovascular system, in both men and women, remain controversial.^{91,92} Testosterone levels fall as men grow older, with a steeper decline in free T compared with total T concentrations.⁹¹ In healthy women, total and free T levels, as well as levels of dehydroepiandrosterone sulfate (DHEAS) and androstenedione, decrease with

age. The steepest decline in androgen levels occurs in the early reproductive years.⁹³ It is generally accepted that androgen levels do not change significantly in the perimenopausal period, although some researchers have challenged this assertion, reporting a decline in T, androstenedione, and SHBG during the menopausal transition.⁹³

Experimental evidence suggests that androgen deficiency contributes to the onset and progression of CVD in men.^{94,95} Androgen deficiency is associated with endothelial dysfunction, adverse lipid profiles, inflammatory responses, altered smooth muscle, and hypertension. Lower T levels have been associated with poor cognitive function, sexual dysfunction, metabolic syndrome, and type 2 diabetes.⁹⁴ These last 2 conditions are associated with CVD. In older men, lower total T levels predict increased incidence of stroke and transient ischemic attacks even after adjusting for conventional CVD risk factors, and observational studies show that blood T concentrations are lower among men with CVD, suggesting a possible preventive role for T therapy.⁹⁵

Androgen treatment may prevent or ameliorate these age-related declines in T.⁹⁶ Short-term effects of various T doses on vascular function have been studied in isolated porcine contracted coronary artery strips. Testosterone produces vasorelaxation of the contracted system in an endothelium-independent manner and without the participation of androgen receptors (ARs). Interestingly, T receptor antagonists flutamide and cyproterone acetate did not interfere with the observed response, again suggesting that the response is independent of ARs.⁹⁷ Indeed, T supplementation restores arterial vasoreactivity, reduces levels of proinflammatory cytokines, cholesterol, and TG, and improves overall endothelial function, although it may also reduce HDL levels.⁹⁸ Human arteries and veins (in both males and females) can locally convert T to estradiol and provide important atheroprotective effects via the mechanisms described above.⁹⁹ It is likely, then, that estradiol's protective vascular effects (either directly due to presence of estrogen or indirectly due to peripheral conversion of androgens) are not restricted to females.

In young women, levels of androgens and estrogens can influence vascular physiology. Polycystic ovarian syndrome (PCOS) and hyperandrogenism are among the most common endocrine disorders in reproductive-age women. Insulin and androgens have complex relationships: a significant number of hyperandrogenic young women have insulin resistance and, conversely, women with diabetes are at greater risk of developing PCOS.¹⁰⁰ From a cardiovascular standpoint, PCOS, hyperandrogenism, and obesity are all associated with a more atherogenic lipid profile.¹⁰⁰ At the time of menopause, women with PCOS have both increased androgen production and increased risk of developing metabolic syndrome compared to their healthy counterparts.¹⁰¹

Despite the previously described studies suggesting a link between hypoandrogenemia and CVD, some researchers have suggested that development of CVD in perimenopausal and postmenopausal with PCOS is related to protracted hyperandrogenism.¹⁰² This assertion is obviously confounded by the multiple other CVR factors present in women with PCOS. In a cohort from the WISE study, a total of 104 postmenopausal women with a history of premenopausal PCOS had more frequent CVD risk factors than women without clinical features of the syndrome, including diabetes, obesity, metabolic syndrome, angiographic coronary disease, and cardiovascular events.¹⁰² Young women with PCOS may benefit from traditional methods of reducing CVD such as calorie restriction, weight loss, and control of blood sugar, pharmacologic methods such as use of sibutramine (an appetite suppressant), or improved control of hyperandrogenism. However, it remains to be determined whether these therapeutic measures may reduce CVR later during postmenopausal years and whether hyperandrogenism in itself is a cause of CVD in PCOS women.

While overall levels of androgens may remain stable in the perimenopausal period, the fall in estrogen levels leads to a decrease in the estradiol/T ratio. This shift seems to be more pronounced in women with increased waist circumference (a surrogate marker of abdominal obesity). This gradual increase in androgenic status may also be implicated in the development of insulin resistance, and a more atherogenic lipoprotein profile, with subsequent increase in CVR after the menopausal transition.¹⁰⁵ In nondiabetic, postmenopausal women who were not using HT, those with CVD displayed lower SHBG levels and free androgen index (FAI) and HbA1c values as compared to controls. Glycosylated hemoglobin values were inversely associated with SHBG, and positively associated with FAI, even after adjusting for age, CVD case control status, and body mass index (BMI). In multivariate models, a significant inverse association between SHBG and HbA1c persisted, as well as a significant positive association between FAI and HbA1c.^{103,104,106}

As previously mentioned, however, other studies have not detected age-adjusted overall differences in androgen levels in postmenopausal women.¹⁰⁷ In a Swedish cohort of perimenopausal women, those with CVD (especially women taking HT) had lower serum androgen levels as compared to matched controls, even when controlled for lipids and other potential risk factors.¹⁰⁸ In addition, in women, there is an inverse relationship between DHEAS, androstenedione, and androgen concentrations and carotid wall thickness as well as between T and SHBG concentrations and carotid atherosclerosis.^{109,110} Endogenous sex hormone levels have also been studied in postmenopausal women undergoing carotid artery endarterectomy. In several studies, an association has been reported between low serum androgen levels and severe internal carotid artery atherosclerosis, suggesting that higher physiological levels have an atherogenic protective role.¹¹¹

Dehydroepiandrosterone (DHEA), in particular, has effects on endothelial proliferation and angiogenesis.¹¹² A specific plasma membrane DHEA receptor has been demonstrated in endothelial cells, and DHEA is metabolized intracellularly to other biologically active steroids, including estradiol (which also induces vascular endothelial proliferation).¹¹² However, T, another DHEA metabolite, does not increase vascular proliferation.¹¹³ In vitro studies indicate that DHEA affects vessels by increasing endothelial NO production. In an acute (and likely non-genomic) fashion, DHEA also increases insulin sensitivity.¹¹⁴ These observations may help explain DHEA's cardioprotective effects. Interestingly, endothelial cells treated with DHEA also increase ET-1 secretion by 2-fold, suggesting that DHEA has both vasodilatory and vasoconstrictive effects on the vasculature.¹¹⁴

ENDOTHELIAL FUNCTION AND THE REGULATION OF VASOMOTOR TONE

The endothelium participates in the control of hemostasis, blood coagulation and fibrinolysis, platelet and leukocyte interactions with the vessel wall and the regulation of vascular tone and blood pressure.¹¹⁵ Endothelial cells produce many compounds aimed at controlling functions of VSMCs and circulating blood cells. In the same individual, endothelial cells from different locations in the vascular system display marked phenotypic variation, expressing different antigens, receptors, and responses to the same stimulus.¹¹⁶ Responses of in vitro cultured endothelial cell may not correspond to those found among the same cells in vivo.^{115,116} Endothelium produces, and is influenced by, a number of vasodilating and vasoconstricting substances that regulate vasomotor tone. Among these mediators are NO, PGI₂, and endothelin. These factors are involved in angiogenesis but may also contribute to atherosclerotic plaque development.¹¹⁶ The biological function of the endothelium is to maintain the vascular network in optimal condition for blood flow.¹¹⁵

The development of atherosclerosis is related, in part, to vascular endothelial dysfunction. The endothelial cell surface maintains a nonthrombogenic blood-tissue interface due to the presence of antithrombin III, thrombomodulin, protein C anticoagulant, plasminogen activator, and PGI₂, and NO (which inhibit platelet activation) and the production of clotting factors.¹¹⁷ A carefully regulated equilibrium between pro- and anticoagulant factors maintains normal blood flow and allows platelet adherence, thrombosis, and thrombolysis.¹¹⁷

Oxidative Stress and NO

Nitric oxide was originally named endothelium-derived relaxing factor.¹¹⁸ It is produced in response to various stimuli, including fluid shear, stress, and exposure to neurohumoral factors such as acetylcholine, bradykinin, serotonin, and substance P. Nitric oxide is formed from the amino acid L-arginine by a 2-step oxidation process involving conversion of L-arginine to L-citrulline, which is catalyzed by eNOS. After its synthesis in the endothelial cell, NO diffuses to the subadjacent VSMCs, where it activates guanylyl cyclase (sGC) and leads to the production of cyclic guanosine monophosphate (cGMP). Although short lived, NO is a potent vasodilator that contributes to the maintenance of basal vascular tone and blood flow and thus to the physiological regulation of blood pressure.¹¹⁸ It also has important antiplatelet, antioxidant, antiadhesive, and antiproliferative properties.^{119,120}

Estrogens can produce rapid vasodilatation by increasing NO synthesis and decreasing ET-1 and angiotensin II production. Estrogen also causes vessel relaxation via acetylcholine- and serotonin-dependent mechanisms, mediated by its effects on PGI₂ secretion and calcium channel inhibition.¹²¹ 17 β estradiol, but not 17 α estradiol, causes rapid endothelial NOS activation. This acute vasodilatory effect occurs via nongenomic pathways involving membrane-associated estrogen binding sites that are independent of nuclear ERs.¹²² In vitro studies have been performed to investigate the effects of progesterone and 17 β estradiol on eNOS in endothelium intact aortic rings. The rapid stimulatory action of progesterone and 17 β estradiol on eNOS (leading to production of NO and ultimately to decreased platelet aggregation) was specific to these 2 hormones; neither T nor 17 α estradiol was effective.¹²³ It seems that nongenomic pathways are important in maintaining normal vasodilatory mechanisms. Transcription of another form of NOS, inducible NOS (iNOS), is positively regulated by ESR2 and negatively regulated by ESR1, suggesting that both receptors are involved in the genomic regulation of vasodilatory mechanisms in VSMC.¹²⁴ ER- β gene knockout mice—deficient in the ER β gene—demonstrate vascular dysfunction and hypertension as a result of iNOS dysregulation.¹²⁵

In response to vasorelaxants, such as acetylcholine, NO acts on underlying VSMCs to induce vascular relaxation. In addition, VSMCs themselves express eNOS and contribute to vascular relaxation.¹²⁶ Endothelial cell incubation with oxidized LDL results in translocation of eNOS to an internal membrane compartment and renders it insensitive to stimulation by acetylcholine.¹²⁷ In addition, elevated HDL is associated with normalization of the endothelium-dependent relaxation response triggered by acetylcholine (via prevention of eNOS translocation).¹²⁸ High-density lipoprotein acts as an atheroprotective substance via direct stimulatory effects on various endothelial kinases that modulate eNOS.^{129,130} Oxidized LDL and HDL thus appear to have opposing effects on endothelial cell NO bioavailability.

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is a broad term that includes a number of proteins from 2 families that result from alternate messenger RNA (mRNA) splicing of a single VEGF gene.¹³¹ They can be proangiogenic (proximal splice site, expressed during

angiogenesis) or antiangiogenic (distal splice site, expressed in normal tissues). The inclusion or exclusion of exons 6 and 7 can modulate VEGF's ability to bind and activate VEGF signaling receptors.¹³¹⁻¹³³

Vascular endothelial growth factor promotes angiogenesis and endothelial cell migration and proliferation via its actions on intracellular mediators.¹³⁴ Although VEGF, as its name implies, acts mainly on vascular endothelial cells, it also affects other cell types (for example, monocytes, macrophages, neurons, and kidney epithelial cells). In vitro, VEGF stimulates endothelial cell mitogenesis and migration. It also enhances vascular permeability—VEGF is 50 000 times more potent than histamine in this regard—and modulates endothelial cell surface adhesion molecule expression.¹³⁵ In regions of increased angiogenesis, VEGF (along with TNF- α and other inflammatory cytokines) is produced by aggregating macrophages.¹³⁶⁻¹³⁹

The VEGF receptor Flt-1 is expressed in human monocytes, which induces chemotactic responses among monocytes.^{140,141} In a strain of ApoE-deficient cholesterol-fed mice, a single dose recombinant human (rh) VEGF treatment significantly increased the number of potentially atherogenic macrophage/monocytes.¹⁴² Therefore, VEGF seems to enhance atherosclerotic plaque progression. However, in patients with ischemic syndromes, intra-arterial rh VEGF administration also markedly increases collateral vessel development.¹⁴³ Thus, VEGF seems to have a potential therapeutic role for coronary and peripheral occlusive vascular disease. Despite this, experimental treatments have failed due to the appearance of endothelial cell angiomatoid proliferation and intima thickening upon treatment with VEGF.¹⁴⁴

Finally, some cardiovascular benefits of the Mediterranean diet have been related to the moderate consumption of red wine, and VEGF may play a role here as well.¹⁴⁵ In cultured VSMCs, short- and long-term exposure to red wine polyphenolic compounds inhibited VEGF expression via inhibition of a specific protein kinase pathway.¹⁴⁶

Prostanoids

Prostanoids, including prostaglandins (PGs) and thromboxanes (TXs), are synthesized from arachidonic acid by the combined action of cyclooxygenases (COXs) and PG and TX synthases.¹⁴⁷ After their synthesis, prostanoids are immediately released into the extracellular space, where they interact with prostanoid receptors on neighboring cells. Prostanoids have potent, although sometimes opposing, biological effects. Prostacyclin is a vasodilator and platelet aggregation inhibitor, whereas TXA₂ acts as a vasoconstrictor and inducer of platelet aggregation.¹⁴⁸ A balance between the 2 is important to maintain cardiovascular health.¹⁴⁷⁻¹⁵⁰

Prostacyclin is the main product of COX (the rate-limiting enzyme in PG biosynthesis) at the vascular endothelium. It is a 20-carbon oxygenated fatty acid, derived from arachidonic acid, which acts as a vasodilator, platelet aggregation inhibitor, and VSMC proliferation inducer.^{151,152} Prostacyclin acts on smooth muscle receptors to activate adenylate cyclase and inhibit vasoconstriction.¹⁵² The PGI₂ pathway, as a vasodilatory system, has some redundancy with the NO system: PGI₂ increases endothelial cell NO production, and NO increases PGI₂ activity on smooth muscle relaxation.¹⁵³ In this capacity, it may function in a compensatory role when other vasorelaxation mechanisms are not functioning (for example, in eNOS-deficient mice who cannot produce NO).¹⁵³ In addition, PGI₂ also inhibits platelet aggregation via inhibition of the platelet-activating factor (PAF), which is synthesized by activated endothelial cells.¹⁵⁴ Interestingly, HDL also has inhibitory effects on endothelial cell PAF in vitro, and HDL particles modulate platelet function in vivo.¹⁵⁵

Thromboxane A₂ is also produced by endothelial cells and serves as a potent vasoconstrictor and platelet activator.¹⁵⁶ It is involved in a wide range of CVDs including hypertension, MI, cerebral vasospasm, preeclampsia, and several thrombotic disorders.¹⁵⁶ In females, it increases vascular tone and blood pressure to compensate for estrogen-sensitive local vasodilating mechanisms and maintain vascular homeostasis.¹⁵⁶ Prostaglandin H synthase (PGHS), an enzyme involved in both PG and TX production, is a target of common nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin and ibuprofen and has been proposed as a possible therapy to prevent plaque formation.^{157,158}

Estrogen interacts with this system at multiple levels. Prostaglandin H synthase-mediated vasodilatation predominates when estrogens are elevated, and estradiol treatment in castrated rats neutralizes PGHS-related vasoconstriction.¹⁵⁹ This finding could be considered a new approach for estrogenic treatment with the potential for further research in human participants.

The link between vascular tone (as regulated by the prostanoid system) and HDL is interesting as well. Prostanoid synthesis is induced by HDL via several mechanisms. Exposure of endothelial cells to HDL in vitro stimulates calcium-sensitive phospholipase A₂, an enzyme required for production of PGI₂.¹⁶⁰ High-density lipoprotein's effect on PGI₂ synthesis can be blocked by incubation of endothelial cells with HDL and calcium chelator.¹⁵⁹ High-density lipoprotein can also act synergistically with the inflammatory cytokines TNF- α and interleukin (IL)-1 β to produce a large increase in COX-2 expression.¹⁶⁰

CARDIOVASCULAR FUNCTION AND THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

Vascular hemostasis does not occur only at the level of the endothelium. Renin-secreting cells at the level of the renal glomerulus are sensitive to changes in blood flow and blood pressure. Renin catalyzes the conversion of the angiotensinogen protein into the decapeptide angiotensin I. Serum angiotensin-converting enzyme (ACE) then converts angiotensin I into the octapeptide angiotensin II that acts via adrenal gland receptors to enhance aldosterone secretion. Aldosterone then stimulates kidney salt and water reabsorption and arteriolar constriction, causing an elevation in blood pressure. Enhanced renin angiotensin aldosterone system (RAAS) activation contributes to the evolution of hypertension.¹⁶¹

Angiogenesis, Endothelial Function, and the RAAS

Angiotensin II acts as an atherogenic agent; it is involved in blood coagulation and the pathogenesis of acute thrombosis via multiple mechanisms. It promotes vascular inflammation, stimulating human endothelial cells to release the inflammatory chemokine interferon inducible protein 10 (IP-10) and potentially promoting atherosclerosis.^{162,163} It also acts on specific membrane receptors to activate intracellular signaling pathways that converge upon the transcription factor nuclear factor (NF)- κ B, resulting in expression of procoagulant tissue factor.¹⁶⁴ Within the atherosclerotic plaque and inflamed vascular endothelium, monocytes and macrophages may synthesize additional angiotensin II, continuing the cycle of coagulation. Angiotensin II has also been shown to induce cellular senescence via a mitogen-activated protein kinase (MAPK) signal pathway.¹⁶⁵

Interestingly, other angiotensin peptide fragments, such as angiotensin IV, may counteract this development of endothelial dysfunction. In ApoE^{-/-} mice fed with a high-fat diet and displaying endothelial dysfunction and impaired vasodilatation, angiotensin IV produced a significant improvement in endothelial function, which seems to be mediated by increased NO bioavailability.¹⁶⁶ Another anti-atherogenic compound, ACE2, is a homologue of ACE

that inactivates the proatherogenic angiotensin II and has been proposed as an atheroprotective substance. In situ hybridization and immunochemistry studies, ACE2 mRNA is expressed in early and advanced human carotid atherosclerotic lesions. During atherosclerosis development, ACE2 levels were lower in stable advanced lesions as compared to early and ruptured atherosclerotic ones.¹⁶⁷

The RAAS appears to cross talk with circulating lipoproteins. For example, some of the protective effects of HDL seem to be mediated by angiotensin II type 1 receptor (AT1R) downregulation, at least under diabetic conditions created by streptozotocin injection. In vivo, 6 weeks after apolipoprotein A-I gene transfer, HDL increase was associated with a 4.7-fold reduction in diabetes mellitus-induced aortic AT1R expression and improvement of NO bioavailability. In vitro, HDL reduced the hyperglycemia-induced upregulation of the AT1R in human aortic endothelial cells.¹⁶⁸ In addition, in fat-fed LDL receptor-deficient (LDLR^{-/-}) mice, the renin inhibitor aliskiren reduces atherosclerotic lesion size in both the aortic arch and the root. When renin-deficient bone marrow is transplanted to irradiated LDLR^{-/-} mice, a profound reduction in the size of atherosclerotic lesions is obtained. Thus, renin inhibition reduces atherosclerotic lesion development.¹⁶⁹

Estrogen's Effects on the RAAS

The RAAS has been implicated in menopause-associated hypertension. In an estrogen-deficient follitropin-receptor knockout mouse model (FORKO), angiotensin II-induced vasoconstriction was enhanced, acetylcholine-induced vasodilatation was suppressed, and blood pressure was elevated. Indices of inflammation (nitrotyrosine formation and superoxide production) and cardiac collagen content were also increased in the FORKO animals.¹⁷⁰

The vasodilator responses of the aortic ring in response to estrogen deprivation have been studied in rats. Animals were assigned to 1 of 3 groups: ovariectomized (OVX) non-OVX (sham) and OVX plus subcutaneous 17 β estradiol (15 μ g/kg per day, OVX + estradiol). Ovariectomized rats had a significant higher blood pressure than the other 2 groups at weeks 9 and 13. A significant decrease in NO levels with increased renin activity was observed in OVX rats as compared to sham operated ones. Estradiol treatment reversed these effects.¹⁷¹ Plasma atrial natriuretic peptide (ANP) levels were also lower in castrated rats and could be restored by estradiol treatment. Acetylcholine-dependent endothelial relaxation was reduced in the isolated thoracic aortic rings of OVX animals, and estradiol treatment restored this response as well. These data supports the conclusion that estradiol is protective to the endothelium, preventing hypertension through modulation of the RAAS.¹⁷¹

Interestingly, however, estrogen treatment increases hepatic renin substrate production in some situations.^{172,173} Hormone therapy increases angiotensin II plasma levels in postmenopausal hypertensive women whose blood pressures were well controlled with antihypertensive agents (excluding diuretics, ACE inhibitors, and angiotensin II receptor antagonists). Angiotensin I, angiotensin II, bradykinin, and renin plasmatic activity manifested a significant increase after HT in hypertensive (but not normotensive) women.¹⁷⁴ However, although angiotensin II levels were increased, blood pressure was unaltered. Serum ACE was significantly decreased in both groups (although aldosterone levels were unchanged), and bradykinin levels were increased (possibly to maintain homeostasis in the setting of elevated angiotensin II).¹⁷⁴ The overall effect of decreased ACE serum activity with increased levels of bradykinin induced by HT may have a cardioprotective effect. In pathological states such as hypertension and congestive cardiac failure, and despite its upregulatory effects on angiotensin II, estrogen may ultimately protect high-risk populations against the potentially deleterious effects of angiotensin II.¹⁷² Hypertension is not considered a contraindication to estrogen treatment.¹⁷⁵

In women with premature ovarian failure, 24-hour systolic and diastolic blood pressures were studied under different HT regimens. In an open-label randomized crossover trial, women with premature ovarian failure were treated with transdermal estradiol and vaginal progesterone or oral standard ethinyl estradiol and norethisterone acetate therapy for 12 months. Both regimens produced the same hormonal response and clinical symptom relief. Combined treatment with transdermal estradiol and vaginal progesterone was associated with lower systolic and diastolic blood pressures than the standard oral regimen at 12 months. In addition, the parenteral/vaginal combination produced a significant reduction in plasma angiotensin II and serum creatinine without altering aldosterone levels.^{175,176}

In a separate study, transdermal HT (estradiol plus medroxyprogesterone acetate or MPA) significantly decreased diastolic and mean blood pressure and bradykinin levels in a group of normotensive postmenopausal women as compared to oral combination HT (continuous oral CEEs plus cyclic oral MPA). There were no significant changes in plasma renin and ACE activity and angiotensin I or angiotensin II levels. In the group of women taking oral HT, after 1 year, blood pressure remained unchanged, renin activity, and angiotensin I, angiotensin II, and bradykinin levels were significantly increased, and ACE activity significantly decreased.¹⁷³ Proper randomized trials are needed to determine whether transdermal HT may be preferable to oral administration of HT with respect to blood pressure and angiotensin II levels.

Aldosteronism is a well-recognized cause of hypertension and also plays a major role in progression and outcome of IHD.¹⁷⁷ Aldosterone is regulated by the pituitary-adrenal axis and the RAAS and itself regulates extracellular fluid content and electrolyte balance. Some new progestins, such as drospirenone, may also have a benefit on perimenopausal women with mild hypertension secondary to hyperaldosteronism.¹⁷⁸

VITAMIN D AND CVR

Vitamin D is a hormone involved in regulating calcium and phosphorus levels that has important autocrine and paracrine roles. It is involved in the maintenance of normal cardiovascular function.¹⁷⁹ The American Heart Association recommends that healthy individuals obtain adequate amounts of vitamin D by consuming a variety of foods in moderation. Such sources include milk, salmon, mackerel, sardines, cod liver oil, and some fortified cereals.¹⁸⁰ A small dose of sunlight may also increase endogenous vitamin D levels without significant skin risks.¹⁸⁰ Vitamin or mineral supplements are not a substitute for a balanced, nutritious diet that limits excessive calories, saturated fat, trans fat, sodium, and dietary cholesterol.¹⁴⁵

Epidemiological, clinical, and experimental data suggest that low serum vitamin D levels are associated with negative cardiovascular outcomes including hypertension, obesity, diabetes mellitus, and the metabolic syndrome.^{181,182} Interestingly, the pleiotropic effects of statins include a stimulatory effect on the vitamin D endocrine system.¹² Vitamin D receptors (VDRs) have a wide tissue distribution that includes VSMCs, endothelium, and cardiomyocytes.¹⁷⁹

The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)₂D), increases VEGF expression (via a vitamin D response element in the VEGF promoter) and release in aortic VSMCs, an effect which, in vitro, is mediated by p38 MAPK activation.¹⁸³ In vitro studies also suggest that vitamin D suppresses proinflammatory cytokines and increases anti-inflammatory cytokines. In patients with congestive heart failure (CHF), vitamin D supplementation increased IL-10 (an anti-inflammatory cytokine) levels after 9 months, while the proinflammatory cytokine TNF- α remained constant. However, survival did not

differ significantly between the individuals who received vitamin D as compared to a control group.¹⁸⁴

A cross-sectional study performed in 257 individuals assessed the relationships between serum 25(OH) D and CVD markers (including HDL levels and prevalence of the metabolic syndrome). Total vitamin D intake, including dietary supplement intake, was directly associated with increased serum vitamin D and HDL-C levels: for each 10 ng/mL serum vitamin D increase, there was a 4.2 mg/dL increase in HDL. Prevalence of the metabolic syndrome decreased significantly as serum vitamin D levels increased.¹⁸⁵ Conversely, low levels of vitamin D also appear to put an individual at risk of CVD. In an analysis of 1739 individuals included in the Framingham Heart Study, those with vitamin D blood levels below 15 ng/mL had twice the risk of a cardiovascular event such as a heart attack, heart failure, or stroke in the next 5.4 years as compared to individuals with higher vitamin D levels. The risk remained significant after adjustments for traditional CVR factors such as high cholesterol, diabetes, and high blood pressure.¹⁷⁹ Endogenous vitamin D levels have also been studied in individuals undergoing coronary angiography. During a median follow-up of 7.7 years, during which 22.6% patients died, patients in the lower two 25-hydroxyvitamin D (25(OH)D) quartiles displayed a higher mortality rate (all-cause and cardiovascular) than those in the highest 25(OH)D serum quartile.¹⁸⁵ Low vitamin D levels also appear to be associated with a higher risk of MI in men aged 40 to 75. Men with blood vitamin D levels <15 ng/mL had a higher risk of infarction than those with higher vitamin D levels (>30 ng/mL). This relationship remained significant even after adjustment for BMI, alcohol consumption, physical activity, history of diabetes mellitus and hypertension, ethnicity, region, omega 3 intake, and lipoprotein levels.¹⁸¹

Finally, in another study, individuals were classified by serum 25(OH)D sex-specific quartile levels. After a mean follow-up period of 6.2 years, 51 participants died, including 20 deaths due to cardiovascular causes. After adjustments for confounding variables such as age, sex, tobacco use, and comorbidities, low 25(OH)D levels were significantly predictive for all-cause (1.97, 1.08-3.58, $P = .027$) and cardiovascular (5.38, 2.02-14.34, $P = .0001$) mortality.¹⁸² However, it remains unclear whether low vitamin D levels are a cause or a consequence of poor cardiovascular health, and more studies are required to confirm the relationship between vitamin D supplementation and reduced mortality.

THE ROLE OF ADIPOCYTE HORMONES

Obesity is associated with increased cardiovascular mortality and morbidity.¹⁸⁶ Obese individuals exhibit vascular endothelial dysfunction, which predicts CVR and is central to the pathogenesis of atherosclerosis.¹⁸⁶ Increased amounts of centrally distributed adipose tissue are associated with increased insulin resistance, type 2 diabetes mellitus, lipid disorders, and CVD.^{187,188} Adipose tissue can be thought of not simply as an energy storage organ but also a secretory organ that produces a variety of bioactive substances capable of influencing the cardiovascular system.¹⁸⁷ There is considerable evidence linking adipocyte hormones and growth factors with cardiovascular complications.¹⁸⁸ Adipose tissue also participates in immunological functions through the production of a number of cytokines that may be involved in the pathogenesis of atherosclerosis, the most important of these adipokines being leptin, adiponectin, visfatin, TNF- α , VEGF, IL-6, resistin, plasminogen activator inhibitor 1 (PAI-1), and angiotensinogen, several of which will be discussed here.¹⁸⁹

Leptin

Leptin is an adipocyte-derived hormone that is released into the blood in direct proportion to the amount of adipose tissue present. It promotes weight loss by decreasing food intake and

increasing metabolic rate. It is also involved in glucose metabolism, as well as in normal sexual maturation and reproduction. Leptin gene expression and secretion are increased by overfeeding, high-fat diet, insulin, glucocorticoids, endotoxins, and cytokines and decreased by fasting, T, thyroid hormone, and exposure to cold.^{186,190}

Leptin interacts with the cardiovascular system in multiple ways. It may play an important role in regulating vascular tone, as evidenced by the widespread distribution of functional leptin receptors in vascular cells.¹⁹⁰ There are also several studies reporting an independent interaction between high leptin levels and atherosclerosis, MI, stroke, and coronary artery intima-media thickness, suggesting that high leptin levels are associated with increased CVR.¹⁹¹⁻¹⁹³ Activation of the sympathetic nervous system by leptin produces a slow but progressive increase in mean arterial blood pressure. In rats, intravenous leptin infusion increased arterial blood pressure and heart rate.¹⁹⁴ Leptin-induced increases in arterial blood pressure is probably mediated by effects on the central nervous system, because intracerebro-ventricular leptin administration is equivalent to systemic administration in terms of its vascular effects.¹⁹⁵

Leptin receptors are also present in endothelial cells, and leptin treatment in rats has been reported to cause dose-dependent increases in NO levels.^{196,197} In vitro studies have also shown that leptin produces endothelium-dependent relaxation of arterial rings.^{198,199} It has been argued that these vasodilatory effects might oppose leptin's neurogenic pressor action. However, these results are controversial; other researchers have not documented leptin-induced increased blood flow in vascular beds.²⁰⁰ Finally, high leptin levels also stimulate superoxide free radical production, which reacts with NO to create peroxynitrite, a toxic molecule that can interfere with DNA replication and damage vascular endothelial cells.²⁰¹ Thus, increased leptin levels can cause long-term cardiovascular damage and possibly contribute to hypertension, atherosclerosis, diabetes and other disorders.

Additionally, in women, circulating leptin levels are associated with stress-induced changes in heart rate, heart rate variability, and cardiac pre-ejection period, independent of age, adiposity, and smoking.²⁰² Plasma leptin levels in women also correlated with stress-induced increased IL-6 levels.²⁰² It has been postulated that leptin may mediate the adverse effects of stress and obesity on female cardiovascular health.

Adiponectin

Adiponectin is an abundant adipocyte-derived plasma protein with anti-atherosclerotic effects.²⁰³ It is a unique adipokine, produced in lower amounts in obese than in lean individuals. Its receptors are present on endothelial cells, and it acts predominantly in a beneficial manner by increasing insulin sensitivity, stimulating fatty acid oxidation, inhibiting inflammatory reactions (including stimulation of the proinflammatory cytokine TNF- α), and inducing endothelium-dependent NO-mediated vasorelaxation.²⁰³⁻²⁰⁵ It is also a negative regulator of angiogenesis: in vitro, addition of adiponectin at physiologic concentrations inhibits endothelial cell proliferation and migration and prevents new vessel growth. It also inhibits nonendothelial cell growth although the concentration needed is higher than for endothelial cells, suggesting a selective action on endothelial cells at low concentrations.²⁰⁶

Adiponectin is an important lipid and glucose metabolism regulator.²⁰⁷ However, in mice, deletion of adiponectin does not cause any differences in body weight, suggesting that, under physiologic conditions, adiponectin may be redundant.²⁰⁸ Similarly, in mice, adiponectin over-expression did not produce a significant increase in body weight or adiposity.²⁰⁵ In humans, high adiponectin concentration has been associated with lower occurrence of diabetes and cholesterol abnormalities. Adiponectin's anti-angiogenic activity

seems to be especially pronounced under pathological conditions and could be related to suppression of angiogenesis that prevents atherosclerotic plaque growth.²⁰⁹ However, despite these metabolic changes, elevated adiponectin levels may lead to increased risk of MI in older adults. In a cohort of 1386 older participants in the population-based Cardiovascular Health Study, 604 suffered a heart disease event, and those with the highest adiponectin levels were the most likely to be affected.²¹⁰ These results would seem to contradict the effects of adiponectin on lipid and glucose regulation, and further research is necessary to clarify adiponectin's role on the cardiovascular system.²¹⁰

Resistin

Resistin is a hormone produced by fat cells that is associated with inflammation and insulin resistance. In a prospective case-control study nested in the Women's Health Study and the Physician's Health Study II, serum resistin levels were significantly higher in postmenopausal women than in men. Elevated baseline resistin levels were associated with an increased risk of type 2 diabetes. Positive correlations have previously been reported between BMI and resistin levels. These findings further suggest a complex interaction between gender, metabolic disease, and inflammatory markers.²¹¹

Resistin exerts a direct effect on myocardial cells, decreasing their ability to contract. In the Health Aging and Body Composition Study, 3000 elderly individuals were followed over 7 years. The risk of new onset heart failure increased 38% for every 10 ng/mL increase in resistin blood levels. This hormone appears to be an even stronger predictor of heart failure risk than other inflammatory markers linked to heart disease, such as C-reactive protein (CRP).²¹² The association of resistin and adiponectin with heart failure has also been studied in 2739 individuals from the Framingham Offspring cohort. The hazard ratio for individuals in the top third of resistin distribution was 4.01 (95% CI: 1.52-10.57) compared to 2.89 (95% CI: 1.05-7.92) in the middle third during 6 years of follow-up. This association remained strong even after adjusting for presence of coronary heart disease, obesity, insulin resistance, and other markers of inflammation.²¹³

Finally, significantly higher resistin levels have been found in individuals with masked hypertension compared to normotensive ones.²¹⁴ These results may be prognostic for future cardiovascular events. Hyperresistinemia may contribute to insulin resistance, endothelial dysfunction, chronic inflammation, and ultimately accelerated atherogenesis.²¹⁵⁻²¹⁷ Therefore, resistin should be included as a novel variable and marker of CVD, although its precise clinical significance remains to be defined.

ADDITIONAL INFLAMMATORY MARKERS

Insulin

Studies of insulin resistance in young populations showed higher fasting insulin concentrations in girls, a difference that has remained despite adjustments for adiposity and pubertal stage.^{218,219} Wilkin and Murphy postulated that females, who are born lighter than males despite higher insulin concentrations at birth, are intrinsically more insulin resistant than males.²²⁰ In women, insulin resistance and diabetes are associated with greater CVR, including up to a 6-fold increase in MI risk, as compared to men (who have a 4-fold increased risk of MI in the setting of diabetes).^{221,222} These observations have been confirmed in multiple studies including the Framingham Heart Project, the Chicago Heart Association Detection Project in Industry, and the Minnesota Heart Survey.^{221,223}

C-Reactive Protein

C-reactive protein is an acute-phase reactant that was originally described in 1930 in the sera of patients acutely ill with pneumococcal pneumonia.²²⁴ The Physicians' Health Study described CRP as a CVD risk factor in men, noting that high plasma concentrations were associated with a 2-fold increased risk of stroke and a 3-fold increased risk of MI.¹⁰⁸ Subsequent studies confirmed its utility as a prognostic factor for CVD in women: an increase in CRP levels above 3.0 mg/L was associated with elevated age-adjusted incidence rates of future cardiovascular events (from 3.4 to 5.9 per thousand person-years of exposure).²²⁵ C-reactive protein does not, however, appear to differ between men and women.²²⁵

Ferritin

Serum ferritin is a biomarker of body iron stores.²²⁶ Sullivan first noted an increased risk of MI in patients with high serum ferritin levels, hypothesizing that elevated iron stores contribute to CVD risk via increased free radical production, which promotes the development of atherosclerosis.^{226,227} While men exhibit higher serum ferritin levels at baseline than both pre- and postmenopausal women, women also appear to be at risk from elevated ferritin levels.²²⁸ In 1 case-cohort study, postmenopausal women with serum ferritin levels in the highest tertile had a 2-fold higher risk of ischemic stroke compared to women in the lowest tertile.²²⁸ These findings are complicated by the fact that other authors have noted a U-shaped association between serum ferritin and CVD, with levels in the extreme low range also representing a CVD factor.²²⁹ In addition, clinical trials testing the impact of phlebotomy on cardiovascular events have not found decreased mortality after reduction of body iron stores.²²⁹ Many of the available studies linking ferritin to CVD risk are small, comprised of varying ethnic groups, and include multiple confounding variables, suggesting that further work needs to be done to clarify the role of ferritin.

Homocysteine

Homocysteine (Hcy) is a sulfur-containing amino acid formed as an intermediate metabolite during the catabolism of the essential amino acid methionine. Some 80% of plasma Hcy is bound to proteins.²³⁰ Its involvement in atherogenesis was elucidated by observing individuals with homozygous homocystinuria (who characteristically present with premature vascular disease).²³⁰ Hyperhomocysteinemia is presently considered an independent risk factor for the development of atherosclerosis, as well as for arterial and venous thrombosis. More than 80 clinical and epidemiological studies support the fact that hyperhomocysteinemia is an atherosclerotic disease risk factor, even among individuals who have normal cholesterol levels.²³¹

Although the precise etiological mechanisms are unknown, hyperhomocysteinemia likely leads to endothelial injury and dysfunction via generation of free radicals.²³² Circulating reactive oxygen species initiate cell membrane and circulating lipoprotein peroxidation. Subsequently, oxidized LDL is taken up by intima macrophages to form foam cells and begin the process of atheromatous plaque formation. The atherogenic effect of Hcy has been confirmed using experimental models; hyperhomocysteinemia can accelerate atherosclerosis development in susceptible models such as the apolipoprotein E-deficient mouse.²³³ Interestingly, however, reduction in Hcy levels does not improve prognosis if disease is already present, as suggested by research studies involving a cohort of nearly 5000 Norwegian heart attack survivors with severe heart disease.²³⁴ No preventive study has yet been conducted among participants who are in a relatively good state of health. In addition, reduction in Hcy levels does not quickly repair existing arterial structural damage.²³⁴

Homocysteine also stimulates VSMC proliferation and collagen deposition in the atheromatous plaque and inhibits vascular endothelial cell growth. Elevated Hcy levels may also promote thrombosis by increased thrombin generation and endothelial cell sensitization to the effect of inflammatory mediators.²³⁵ Other possible mechanisms for Hcy-mediated atherogenesis include decreased NO bioavailability and excessive endothelial monocyte/neutrophil adhesion.²³⁶ Evidence from animal models has demonstrated that hyperhomocysteinemia stimulates vascular cell proinflammatory pathways, resulting in vessel wall leukocyte recruitment and infiltration with increased chemokine secretion, and monocyte differentiation into cholesterol scavenging macrophages.²³⁵ Finally, accumulation of adenosylhomocysteine (a by-product of hyperhomocysteinemia) leads to inhibition of methyltransferases and ultimately prevents repair of aged and damaged cells.²³⁷

In vitro experimental results indicate that Hcy can lead to ESR1 hypermethylation. This process is correlated with more severe atherosclerotic lesions; patients with atherosclerosis have a high rate of ESR1 promoter region hypermethylation.²³⁷ Interestingly, menopausal women who took micronized estradiol (2 mg/d) for 6 months, with or without the addition of norethisterone acetate (1 mg/d), had a significant reduction in Hcy levels. However, there was no correlation between Hcy levels and measurements of carotid vascular resistance following HT.²³⁸ In men, there were no associations between Hcy and T, DHEAS, and estradiol levels, even after adjustments for smoking, alcohol intake, daily physical activity, diabetes mellitus, and hypertension.²³⁹

FINAL REMARKS

Cardiovascular disease is a complex process that includes genetic, inflammatory, and immune factors as well as endocrine components. During the last decades, much emphasis has been given to blood cholesterol and lipids as the primary determinants of CVR. However, many endocrine factors (including androgens and estrogens) and biochemical factors are involved in the atherosclerosis process as well, both systemically and at the level of the vascular endothelium. Much research remains to be done regarding the interaction between these various factors and their role in gender-related cardiovascular differences.

Acknowledgments

This work has been partially supported by the B/017543/08 AECID ("Agencia Española de Cooperación Internacional para el Desarrollo") grant from the Spanish "Ministerio de Asuntos Exteriores y Cooperación." The authors declare no conflicts of interest related to this work or publication.

REFERENCES

1. Okey R, Stewart D. Diet and blood cholesterol in normal women. *J Biol Chem.* 1932; 99(3):717, 1933.
2. Anonymous. Hormones and atherosclerosis. Meeting in Utah. *BMJ.* 1958; 1(5078):1059–1060.
3. Bonneux L, Barendregt JJ. Ischaemic heart disease and cholesterol. There's more to heart disease than cholesterol. *BMJ.* 1994; 308(6935):1038, 1041. [PubMed: 7710473]
4. Ravnskov U. Ischaemic heart disease and cholesterol. Optimism about drug treatment is unjustified. *BMJ.* 1994; 308(6935):1038, 1041. [PubMed: 8167521]
5. Marín A, Medrano MJ, González J, et al. Risk of ischaemic heart disease and acute myocardial infarction in a Spanish population: observational prospective study in a primarycare setting. *BMC Public Health.* 2006; 6:38. [PubMed: 16503965]
6. Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *BMJ.* 2003; 326(7404):1423. [PubMed: 12829554]

7. Brunzell JD. Clinical practice. Hypertriglyceridemia. *N Engl J Med*. 2007; 357(10):1009–1017. [PubMed: 17804845]
8. Lieb W, Larson MG, Benjamin EJ, et al. Multimarker approach to evaluate correlates of vascular stiffness: the Framingham Heart Study. *Circulation*. 2009; 119(1):37–43. [PubMed: 19103986]
9. Ridker PM, Danielson E, Fonseca FA, et al. for the JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008; 359(21): 2195–2207. [PubMed: 18997196]
10. Glynn RJ, MacFadyen JG, Ridker PM. Tracking of high-sensitivity C-reactive protein after an initially elevated concentration: the JUPITER Study. *Clin Chem*. 2009; 55(2):305–312. [PubMed: 19095726]
11. González-Clemente JM, Giménez-Palop O, Vilardell C, Caixàs A, Giménez-Pérez G. Are statins analogues of vitamin D? *Lancet*. 2006; 368(9543):1233.
12. Pérez-Castrillón JL, Vega G, Abad L, et al. Effects of atorvastatin on vitamin D levels in patients with acute ischemic heart disease. *Am J Cardiol*. 2007; 99(7):903–905. [PubMed: 17398180]
13. Penttinen J. Hypothesis: low serum cholesterol, suicide, and interleukin-2. *Am J Epidemiol*. 1995; 141(8):716–718. [PubMed: 7709913]
14. Hillbrand M, Waite BM, Miller DS, Spitz RT, Lingswiler VM. Serum cholesterol concentrations and mood states in violent psychiatric patients: an experience sampling study. *J Behav Med*. 2000; 23(6):519–529. [PubMed: 11199085]
15. Marcoff L, Thompson PD. The role of coenzyme Q10 in statin-associated myopathy: a systematic review. *J Am Coll Cardiol*. 2007; 49(23):2231–2237. [PubMed: 17560286]
16. Phillips GB. Is atherosclerotic cardiovascular disease an endocrinological disorder? The estrogen-androgen paradox. *J Clin Endocrin Metab*. 2005; 90(5):2708–2711.
17. Wannamethee SG, Tchernova J, Whincup P, et al. Plasma leptin: associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. *Atherosclerosis*. 2007; 191(2):418–426. [PubMed: 16712853]
18. Michos ED, Melamed ML. Vitamin D and cardiovascular disease risk. *Curr Opin Clin Nutr Metab Care*. 2008; 11(1):7–12. [PubMed: 18090651]
19. Pérez-López FR. Vitamin D metabolism and cardiovascular risk factors in postmenopausal women. *Maturitas*. 2009; 62(3):248–262. [PubMed: 19211206]
20. Perez-Lopez FR, Chedraui P, Gilbert J, Perez-Roncero G. Cardiovascular risk in menopausal women and prevalent related comorbid conditions: facing the post-WHI era. *Fertil Steril*. 2009; 92(4):1171–1186. [PubMed: 19700149]
21. Kinder L, Carnethon M, Palaniappan L, King A, Fortmann S. Depression and the metabolic syndrome in young adults: findings from the National Health and Nutrition Examination Survey. *Psychosom Med*. 2004; 66(3):316–322. [PubMed: 15184689]
22. Fatourehchi V. Subclinical hypothyroidism: an update for primary care physicians. *Mayo Clin Proc*. 2009; 84(1):65–71. [PubMed: 19121255]
23. Evangelista O, McLaughlin M. Review of cardiovascular risk factors in women. *Gend Med*. 2009; 6(1):17–36. [PubMed: 19318217]
24. Mankad R, Best P. Cardiovascular disease in older women: a challenge in diagnosis and treatment. *Womens Health*. 2008; 4(5):449–464.
25. Kaseta JR, Skafar DF, Ram JL, Jacober SJ, Sowers JR. Cardiovascular disease in the diabetic woman. *J Clin Endocrinol Metab*. 1999; 84(6):1835–1838. [PubMed: 10372670]
26. Lorenzo C, Williams K, Hunt KJ, Haffner SM. The National Cholesterol Education Program—Adult Treatment Panel III, International Diabetes Federation, and World Health Organization definitions of the metabolic syndrome as predictors of incident cardiovascular disease and diabetes. *Diabetes Care*. 2007; 30(1):8–13. [PubMed: 17192325]
27. Shearman AM, Cooper JA, Kotwinski PJ, et al. Estrogen receptor alpha gene variation is associated with risk of myocardial infarction in more than seven thousand men from five cohorts. *Circ Res*. 2006; 98(5):590–592. [PubMed: 16484614]
28. Hodges YK, Tung L, Yan XD, Graham JD, Horwitz KB, Horwitz LD. Estrogen receptors alpha and beta: prevalence of estrogen receptor beta mRNA in human vascular smooth muscle and transcriptional effects. *Circulation*. 2000; 101(15):1792–1798. [PubMed: 10769279]

29. Schuit SC, Oei HH, Witteman JC, et al. Estrogen receptor alpha gene polymorphisms and risk of myocardial infarction. *JAMA*. 2004; 291(24):2969–2977. [PubMed: 15213208]
30. Schuit SC, de Jong FH, Stolk L, et al. Estrogen receptor alpha gene polymorphisms are associated with estradiol levels in postmenopausal women. *Eur J Endocrinol*. 2005; 153(2):327–334. [PubMed: 16061840]
31. Liu PY, Christian RC, Ruan M, Miller VM, Fitzpatrick LA. Correlating androgen and estrogen steroid receptor expression with coronary calcification and atherosclerosis in men without known coronary artery disease. *J Clin Endocrinol Metab*. 2005; 90(2):1041–1046. [PubMed: 15536156]
32. Christian RC, Liu PY, Harrington S, Ruan M, Miller VM, Fitzpatrick LA. Intimal estrogen receptor (ER) beta, but not ER alpha expression, is correlated with coronary calcification and atherosclerosis in pre- and postmenopausal women. *J Clin Endocrinol Metab*. 2006; 91(7):2713–2720. [PubMed: 16608893]
33. Lindner V, Kim SK, Karas RH, Kuiper GG, Gustafsson JA, Mendelsohn ME. Increased expression of estrogen receptor-beta mRNA in male blood vessels after vascular injury. *Circ Res*. 1998; 83(2):224–229. [PubMed: 9686763]
34. Rexrode KM, Ridker PM, Hegener HH, Buring JE, Manson JAE, Zee RYL. Polymorphisms and haplotypes of the estrogen receptor- β gene (*ESR2*) and cardiovascular disease in men and women. *Clin Chem*. 2007; 53(10):1749–1756. [PubMed: 17702854]
35. Domingues-Montanari S, Subirana I, Tomás M, Marrugat J, Sentí M. Association between *ESR2* genetic variants and risk of myocardial infarction. *Clin Chem*. 2008; 54(7):1183–1189. [PubMed: 18487282]
36. Goulart AC, Zee RY, Rexrode KM. Association of estrogen receptor 2 gene polymorphisms with obesity in women (obesity and estrogen receptor 2 gene). *Maturitas*. 2009; 62(2):179–183. [PubMed: 19111408]
37. Haas E, Bhattacharya I, Brailoiu E, et al. Regulatory role of G protein-coupled estrogen receptor for vascular function and obesity. *Circ Res*. 2009; 104(3):288–291. [PubMed: 19179659]
38. Mårtensson UE, Salehi SA, Windahl S, et al. Deletion of the G protein-coupled receptor 30 impairs glucose tolerance, reduces bone growth, increases blood pressure, and eliminates estradiol-stimulated insulin release in female mice. *Endocrinology*. 2009; 150(2):687–698. [PubMed: 18845638]
39. Barrett-Connor E, Bush TL. Estrogens and coronary heart disease in women. *JAMA*. 1991; 265(14):1861–1867. [PubMed: 2005736]
40. Reckelhoff JF. Gender differences in the regulation of blood pressure. *Hypertension*. 2001; 37(5):1199–1208. [PubMed: 11358929]
41. Ho JE, Paultre F, Mosca L. The gender gap in coronary heart disease mortality: is there a difference between blacks and whites? *J Womens Health (Larchmt)*. 2005; 14(2):117–127. [PubMed: 15775729]
42. Perez Lopez, F.; Cuadros-Lopez, J.; Fernandez, A.; Cuadros-Celorrio, A.; Sabatel-Lopez, R.; Chedraui, P. Assessing fatal cardiovascular disease with the SCORE scale in postmenopausal women 10 years after different hormonal treatment regimens.. *Gynecological Endocrinology*. <http://www.gynecologicalendocrinology.org/services/journal.htm>. Posted online on 17 Nov 2009
43. Lemieux S, Després JP, Moorjani S, et al. Are gender differences in cardiovascular disease risk factors explained by the level of visceral adipose tissue? *Diabetologia*. 1994; 37(8):757–764. [PubMed: 7988777]
44. Tomaszewski M, Charchar FJ, Maric C, et al. Association between lipid profile and circulating concentrations of estrogens in young men. *Atherosclerosis*. 2009; 203(1):257–262. [PubMed: 18639879]
45. Mohlke KL, Boehnke M, Abecasis GR. Metabolic and cardiovascular traits: an abundance of recently identified common genetic variants. *Hum Mol Genet*. 2008; 17(R2):R102–R108. [PubMed: 18852197]
46. Okada M, Nomura S, Ikoma Y, Yamamoto E, Ito T, Matsui T, Tamakoshi K, Mizutani S. Effects of postmenopausal hormone therapy on hemoglobin A1C levels. *Diabetes Care*. 2003; 26(4):1088–1092. [PubMed: 12663578]

47. Johnson J, Slentz C, Duscha B, et al. Gender and racial differences in lipoprotein subclass distributions: the STRRIDE study. *Atherosclerosis*. 2004; 176(2):371–377. [PubMed: 15380461]
48. Otvos JD, Collins D, Freedman DS, et al. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Circulation*. 2006; 113(12):1556–1563. [PubMed: 16534013]
49. Persson J, Nilsson J, Lindholm MW. Cytokine response to lipoprotein lipid loading in human monocyte-derived macrophages. *Lipids Health Dis*. 2006; 5:17. [PubMed: 16800873]
50. Rifici VA, Khachadurian AK. The inhibition of low-density lipoprotein oxidation by 17 β -estradiol. *Metabolism*. 1992; 41(10):1110–1114. [PubMed: 1328822]
51. Negre-Salvayre A, Pieraggi MT, Mabile L, Salvayre R. Protective effect of 17- β -estradiol against the cytotoxicity of minimally oxidized LDL to cultured bovine aortic endothelial cells. *Atherosclerosis*. 1993; 99(2):209–217.
52. Huber LA, Scheffler E, Poll T, Ziegler R, Dresel HA. 17 Beta-estradiol inhibits LDL oxidation and cholesterol ester formation in cultured macrophages. *Free Radic Res Commun*. 1990; 8(3):167–173. [PubMed: 2328927]
53. Lloyd-Jones DM, O'Donnell CJ, D'Agostino RB, Massaro J, Silbershatz H, Wilson PW. Applicability of cholesterol-lowering primary prevention trials to a general population: the Framingham heart study. *Arch Intern Med*. 2001; 161(7):949–954. [PubMed: 11295957]
54. Third Report of the National Cholesterol Education Program (NCEP). Detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *Circulation*. 2002; 106(25):3143–3421. [PubMed: 12485966]
55. Shlipak MG, Simon JA, Vittinghoff E, et al. Estrogen and progestin, lipoprotein(a) and the risk of recurrent coronary heart disease events after menopause. *JAMA*. 2000; 283(14):1845–1852. [PubMed: 10770146]
56. Vaidya D, Dobs A, Gapstur SM, et al. The association of endogenous sex hormones with lipoprotein subfraction profile in the Multi-Ethnic Study of Atherosclerosis. *Metabolism*. 2008; 57(6):782–790. [PubMed: 18502260]
57. Freedman DS, Otvos JD, Jeyarajah EJ, et al. Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. *Clin Chem*. 2004; 50(7):1189–1200. [PubMed: 15107310]
58. Rossouw JE, Anderson GL, Prentice RL, et al. for the Writing Group for the Women's Health Initiative Investigators. 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(3):321–333. [PubMed: 12117397]
59. Anderson G, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the WHI RCT. *JAMA*. 2004; 291(14):1701–1712. [PubMed: 15082697]
60. Turgeon J, McDonnell D, Martin K, Wise P. Hormone therapy: physiological complexity belies therapeutic simplicity. *Science*. 2004; 304(5676):1269–1273. [PubMed: 15166356]
61. Naftolin F, Taylor HS, Karas R, et al. for the Women's Health Initiative. The Women's Health Initiative could not have detected cardioprotective effects of starting hormone therapy during the menopausal transition. *Fertil Steril*. 2004; 81(6):1498–1501. [PubMed: 15193467]
62. Manson JE, Allison MA, Rossouw JE, et al. Estrogen therapy and coronary-artery calcification. *N Engl J Med*. 2007; 356(25):2591–2602. [PubMed: 17582069]
63. Egbrink MG, Van Gestel MA, Broeders MA, et al. Regulation of microvascular thromboembolism in vivo. *Microcirculation*. 2005; 12(3):287–300. [PubMed: 15814437]
64. Maffei S, Mercuri A, Prontera C, Zucchelli GC, Vassalle C. Vasoactive biomarkers and oxidative stress in healthy recently postmenopausal women treated with hormone replacement therapy. *Climacteric*. 2006; 9(6):452–458. [PubMed: 17085378]
65. Taddei S, Virdis A, Ghiadoni L, Versari D, Salvetti A. Endothelium, aging, and hypertension. *Curr Hypertens Rep*. 2006; 8(1):84–89. [PubMed: 16600164]
66. Byar DP. Proceedings: the Veterans Administration Cooperative Urological Research Group's studies of cancer of the prostate. *Cancer*. 1973; 32(5):1126–1130. [PubMed: 4585929]

67. Akkad AA, Halligan AW, Abrams K, al-Azzawi F. Differing responses in blood pressure over 24 hours in normotensive women receiving oral or transdermal estrogen replacement therapy. *Obstet Gynecol.* 1997; 89(1):97–103. [PubMed: 8990447]
68. Harmon S, Brinton E, Cedars M, et al. KEEPS: The Kronos Early Estrogen Prevention Study. *Climacteric.* 2005; 8(1):3–12.
69. Farhat MY, Lavigne MC, Ramwell PW. The vascular protective effects of estrogen. *FASEB J.* 1996; 10(5):615–624. [PubMed: 8621060]
70. Skafar DF, Xu R, Morales J, Ram J, Sowers JR. Female sex hormones and cardiovascular disease in women. *J Clin Endocrinol Metab.* 1997; 82(12):3913–3918. [PubMed: 9398686]
71. Simoncini T, Mannella P, Fornari L, et al. Differential signal transduction of progesterone and medroxyprogesterone acetate in human endothelial cells. *Endocrinology.* 2004; 145(12):5745–5756. [PubMed: 15358673]
72. Kublickiene K, Svedas E, Landgren BM, et al. Small artery endothelial dysfunction in postmenopausal women: in vitro function, morphology, and modification by estrogen and selective estrogen receptor modulators. *J Clin Endocrinol Metab.* 2005; 90(11):6113–6122. [PubMed: 16131583]
73. Rossouw JE, Prentice PL, Manson JE, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA.* 2007; 297(13):1465–1477. [PubMed: 17405972]
74. Rivera CM, Grossardt BR, Rhodes DJ, et al. Increased cardiovascular mortality after early bilateral oophorectomy. *Menopause.* 2009; 16(1):15–23. [PubMed: 19034050]
75. Nelson LM. Primary ovarian insufficiency. *N Engl J Med.* 2009; 360(6):606–614. [PubMed: 19196677]
76. Parker WH, Manson JE. Oophorectomy and cardiovascular mortality: is there a link? *Menopause.* 2009; 16(1):1–2. [PubMed: 18971791]
77. Collins P. Vascular effect of hormones. *Maturitas.* 2001; 38(1):45–51. [PubMed: 11311587]
78. Schnoes KK, Jaffe IZ, Iyer L, et al. Rapid recruitment of temporally distinct vascular gene sets by estrogen. *Mol Endocrinol.* 2008; 22(11):2544–2556. [PubMed: 18787042]
79. Peter I, Kelley-Hedgpeath A, Huggins GS, et al. Association between arterial stiffness and variations in oestrogen-related genes. *J Hum Hypertens.* 2009; 23(10):636–644. [PubMed: 19194457]
80. Williams JK, Delansorne R, Paris J. Estrogens, progestins and coronary artery reactivity in atherosclerotic monkeys. *J Steroid Biochem Mol Biol.* 1998; 65(1-6):219–224. [PubMed: 9699876]
81. Mendelsohn ME. Genomic and nongenomic effects of estrogen in the vasculature. *Am J Cardiol.* 2002; 90(1A):3F–6F.
82. Teoh H, Quan A, Leung SW, Man RY. Vascular effects of estrone and diethylstilbestrol in porcine coronary arteries. *Menopause.* 2009; 16(1):104–109. [PubMed: 19131845]
83. Mercurio G, Zoncu S, Saiu F, Mascia M, Melis GB, Rosano GM. Menopause induced by oophorectomy reveals a role of ovarian estrogen on the maintenance of pressure homeostasis. *Maturitas.* 2004; 47(2):131–138. [PubMed: 14757272]
84. Ichikawa J, Sumino H, Ichikawa S, Ozaki M. Different effects of transdermal and oral hormone replacement therapy on the renin-angiotensin system, plasma bradykinin level, and blood pressure of normotensive postmenopausal women. *Am J Hypertens.* 2006; 19(7):744–749. [PubMed: 16814131]
85. Mueck AO, Seeger H. Effect of hormone therapy on BP in normotensive and hypertensive postmenopausal women. *Maturitas.* 2004; 49(3):189–203. [PubMed: 15488347]
86. Vongpatanasin W, Tuncel M, Mansour Y, Arbique D, Victor RG. Transdermal estrogen replacement therapy decreases sympathetic activity in postmenopausal women. *Circulation.* 2001; 103(24):2903–2908. [PubMed: 11413078]
87. Haas E, Meyer MR, Schurr U, et al. Differential effects of 17beta-estradiol on function and expression of estrogen receptor alpha, estrogen receptor beta, and GPR30 in arteries and veins of patients with atherosclerosis. *Hypertension.* 2007; 49(6):1358–1363. [PubMed: 17452498]

88. Tech H, Quan A, Leung S. Vascular effects of estrone and DES in porcine coronary arteries. *Menopause*. 2009; 16(1):104–109. [PubMed: 19131845]
89. Barton M, Cremer J, Mügge A. 17Beta-estradiol acutely improves endothelium-dependent relaxation to bradykinin in isolated human coronary arteries. *Eur J Pharmacol*. 1998; 362(1):73–76. [PubMed: 9865533]
90. Lewis DA, Avsar M, Labreche P, Bracamonte M, Jayachandran M, Miller VM. Treatment with raloxifene and 17beta-estradiol differentially modulates nitric oxide and prostanoids in venous endothelium and platelets of ovariectomized pigs. *J Cardiovasc Pharmacol*. 2006; 48(5):231–238. [PubMed: 17110805]
91. Liu PY, Death AK, Handelsman DJ. Androgens and cardiovascular disease. *Endocr Rev*. 2003; 24(3):313–340. [PubMed: 12788802]
92. Rexrode KM, Ridker PM, Hegener HH, Buring JE, Manson JE, Zee RY. Genetic variation of the androgen receptor and risk of myocardial infarction and ischemic stroke in women. *Stroke*. 2008; 39(5):1590–1592. [PubMed: 18323476]
93. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: Changes with age, menopause, and oophorectomy. *J Clin Endocrinol Metab*. 2005; 90(7):3847–3853. [PubMed: 15827095]
94. Yeap BB. Are declining testosterone levels a major risk factor for ill-health in aging men? *Int J Impot Res*. 2009; 21(1):24–36. [PubMed: 19037223]
95. 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(7):2353–2359. [PubMed: 19351733]
96. 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(6):4055–4060. [PubMed: 11904449]
97. Teoh H, Quan A, Leung SW, Man RY. Differential effects of 17beta-estradiol and testosterone on the contractile responses of porcine coronary arteries. *Br J Pharmacol*. 2000; 129(7):1301–1308. [PubMed: 10742284]
98. Traish AM, Saad F, Feeley RJ, Guay AT. The dark side of testosterone deficiency: III. Cardiovascular disease. *J Androl*. 2009; 30(5):477–494. [PubMed: 19342698]
99. Diano S, Horvath TL, Mor G, et al. Aromatase and estrogen receptor immunoreactivity in the coronary arteries of monkeys and human subjects. *Menopause*. 1999; 6(1):21–28. [PubMed: 10100176]
100. Valkenburg O, Steegers-Theunissen RP, Smedts HP, et al. A more atherogenic serum lipoprotein profile is present in women with polycystic ovary syndrome: a case-control study. *J Clin Endocrinol Metab*. 2008; 93(2):470–476. [PubMed: 18056772]
101. Luque-Ramírez M, Mendieta-Azcona C, Alvarez-Blasco F, Escobar-Morreale HF. Androgen excess is associated with the increased carotid intima-media thickness observed in young women with polycystic ovary syndrome. *Hum Reprod*. 2007; 22(12):3197–3203. [PubMed: 17933750]
102. Shaw LJ, Bairey Merz CN, Azziz R, et al. Postmenopausal women with a history of irregular menses and elevated androgen measurements at high risk for worsening cardiovascular event-free survival: results from the National Institutes of Health—National Heart, Lung, and Blood Institute sponsored Women's Ischemia Syndrome Evaluation. *J Clin Endocrinol Metab*. 2008; 93(4):1276–1284. [PubMed: 18182456]
103. Florakis D, Diamanti-Kandarakis E, Katsikis I, et al. Effect of hypocaloric diet plus sibutramine treatment on hormonal and metabolic features in overweight and obese women with polycystic ovary syndrome: a randomized, 24-week study. *Int J Obes (Lond)*. 2008; 32(4):692–699. [PubMed: 18071341]
104. Maturana MA, Breda V, Lhullier F, Spritzer PM. Relationship between endogenous testosterone and cardiovascular risk in early postmenopausal women. *Metabolism*. 2008; 57(7):961–965. [PubMed: 18555838]
105. Barrett-Connor EL, Goodman-Gruen D. Prospective study of endogenous sex hormones and fatal cardiovascular disease in postmenopausal women. *Br Med J*. 1995; 311(7014):1193–1196. [PubMed: 7488894]

106. Ding EL, Song Y, Manson JE, Rifai N, Buring JE, Liu S. Plasma sex steroid hormones and risk of developing type 2 diabetes in women: a prospective study. *Diabetologia*. 2007; 50(10):2076–2084. [PubMed: 17701157]
107. Khatibi A, Agardh CD, Shakir YA, et al. Could androgens protect middle-aged women from cardiovascular events? A population-based study of Swedish women: The Women's Health in the Lund Area (WHILA) Study. *Climacteric*. 2007; 10(5):386–392. [PubMed: 17852141]
108. Page G, Goulart A, Rexrode K. Interrelation between sex hormones and plasma SHBG and HbA1C in healthy postmenopausal women. *Metab Syndr Relat Disord*. 2009; 7(3):249–254. [PubMed: 19344226]
109. Bernini GP, Moretti A, Sgró M, et al. Influence of endogenous androgens on carotid wall in postmenopausal women. *Menopause*. 2001; 8(1):43–50. [PubMed: 11201514]
110. Golden SH, Maguire A, Ding J, et al. Endogenous postmenopausal hormones and carotid atherosclerosis: a case-control study of the atherosclerosis risk in communities cohort. *Am J Epidemiol*. 2002; 155(5):437–445. [PubMed: 11867355]
111. Debing E, Peeters E, Duquet W, Poppe K, Velkeniers B, Van den Brande P. Endogenous sex hormone levels in postmenopausal women undergoing carotid artery endarterectomy. *Eur J Endocrinol*. 2007; 156(6):687–693. [PubMed: 17535869]
112. Liu D, Dillon JS. Dehydroepiandrosterone stimulates nitric oxide release in vascular endothelial cells: evidence for a cell surface receptor. *Steroids*. 2004; 69(4):279–289. [PubMed: 15183694]
113. Ling S, Dai A, Williams MR, et al. Testosterone (T) enhances apoptosis-related damage in human vascular endothelial cells. *Endocrinology*. 2002; 143(3):1119–1125. [PubMed: 11861539]
114. Formoso G, Chen H, Kim JA, Montagnani M, Consoli A, Quon MJ. Dehydroepiandrosterone mimics acute actions of insulin to stimulate production of both nitric oxide and endothelin 1 via distinct phosphatidylinositol 3-kinase- and mitogen-activated protein kinase-dependent pathways in vascular endothelium. *Mol Endocrinol*. 2006; 20(5):1153–1163. [PubMed: 16373398]
115. Galley HF, Webster NR. Physiology of the endothelium. *Brit J Anaesth*. 2004; 93(1):105–113. [PubMed: 15121728]
116. Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. *N Engl J Med*. 1990; 323(1):27–36. [PubMed: 2113184]
117. Thambyrajah J, Townend JN. Homocysteine and atherothrombosis—mechanisms for injury. *Eur Heart J*. 2000; 21(12):967–974. [PubMed: 10901508]
118. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet*. 1989; 2(8670):997–1000. [PubMed: 2572793]
119. Sase K, Michel T. Expression of constitutive endothelial nitric oxide synthase in human blood platelets. *Life Sci*. 1995; 57(22):2049–2055. [PubMed: 7475956]
120. Chen LY, Mehta JL. Further evidence of the presence of constitutive and inducible nitric oxide synthase isoforms in human platelets. *J Cardiovasc Pharmacol*. 1996; 27(1):154–158. [PubMed: 8656650]
121. Arnal JF, Douin-Echinard V, Bouchet L, et al. Understanding the oestrogen action in experimental and clinical atherosclerosis. *Fundam Clin Pharmacol*. 2006; 20(6):539–548. [PubMed: 17109647]
122. Hisamoto K, Ohmichi M, Kurachi H, et al. Estrogen induces the Akt-dependent activation of endothelial nitric-oxide synthase in vascular endothelial cells. *J Biol Chem*. 2001; 276(5):3459–3467. [PubMed: 11044445]
123. Selles J, Polini N, Alvarez C, Massheimer V. Progesterone and 17 beta-estradiol acutely stimulate nitric oxide synthase activity in rat aorta and inhibit platelet aggregation. *Life Sci*. 2001; 69(7):815–827. [PubMed: 11487093]
124. Tsutsumi S, Zhang X, Takata K, et al. Differential regulation of the inducible nitric oxide synthase gene by estrogen receptors 1 and 2. *J Endocrinol*. 2008; 199(2):267–273. [PubMed: 18753331]
125. Zhu Y, Bian Z, Lu P, et al. Abnormal vascular function and hypertension in mice deficient in estrogen receptor β . *Science*. 2002; 295(5554):505–508. [PubMed: 11799247]

126. Buchwalow IB, Cacanyiova S, Neumann J, Samoilova VE, Boecker W, Kristek F. The role of arterial smooth muscle in vasorelaxation. *Biochem Biophys Res Commun*. 2008; 377(2):504–507. [PubMed: 18929540]
127. Vita JA, Treasure CB, Nabel EG, et al. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. *Circulation*. 1990; 81(2):491–497. [PubMed: 2105174]
128. Watts GF, O'Brien SF, Silvester W, Millar JA. Impaired endothelium-dependent and independent dilatation of forearm resistance arteries in men with diet-treated non-insulin-dependent diabetes: role of dyslipidaemia. *Clin Sci (Colch)*. 1996; 91(5):567–573. [PubMed: 8942395]
129. Uittenbogaard A, Shaul PW, Yuhanna IS, Blair A, Smart EJ. High density lipoprotein prevents oxidized low density lipoprotein-induced inhibition of endothelial nitric-oxide synthase localization and activation in caveolae. *J Biol Chem*. 2000; 275(15):11278–11283. [PubMed: 10753938]
130. O'Conner BJ, Genest J Jr. High-density lipoproteins and endothelial function. *Circulation*. 2001; 104(16):1978–1983. [PubMed: 11602504]
131. Ferrara N, Gerber HP. The role of vascular endothelial growth factor in angiogenesis. *Acta Haematol*. 2002; 106(4):148–156. [PubMed: 11815711]
132. Matsumoto T, Mugishima. Signal transduction via vascular endothelial growth factor (VEGF) receptors and their roles in atherogenesis. *J Atheroscler Thromb*. 2006; 13(3):130–135. [PubMed: 16835467]
133. Shibuya M. Differential roles of vascular endothelial growth factor receptor-1 and receptor-2 in angiogenesis. *J Biochem Mol Biol*. 2006; 39(5):469–478. [PubMed: 17002866]
134. Shizukuda Y, Tang S, Yokota R, Ware JA. Vascular endothelial growth factor-induced endothelial cell migration and proliferation depend on a nitric oxide-mediated decrease in protein kinase Cdelta activity. *Circ Res*. 1999; 85(3):247–256. [PubMed: 10436167]
135. Lal BK, Varma S, Pappas PJ, Hobson RW 2nd, Durán WN. VEGF increases permeability of the endothelial cell monolayer by activation of PKB/akt, endothelial nitric-oxide synthase, and MAP kinase pathways. *Microvasc Res*. 2001; 62(3):252–262. [PubMed: 11678628]
136. Leek RD, Hunt NC, Landers RJ, Lewis CE, Royds JA, Harris AL. Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer. *J Pathol*. 2000; 190(4):430–436. [PubMed: 10699991]
137. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med*. 1999; 340(2):115–126. [PubMed: 9887164]
138. Jonasson L, Holm J, Skalli O, Bondjers G, Hansson GK. Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. *Arteriosclerosis*. 1986; 6(2):131–138. [PubMed: 2937395]
139. Häkkinen T, Karkola K, Ylä-Herttuala S. Macrophages, smooth muscle cells, endothelial cells, and T-cells express CD40 and CD40L in fatty streaks and more advanced human atherosclerotic lesions. Colocalization with epitopes of oxidized low-density lipoprotein, scavenger receptor, and CD16 (Fc gammaRIII). *Virchows Arch*. 2000; 437(4):396–405. [PubMed: 11097365]
140. Clauss M, Gerlach M, Gerlach H, et al. Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cells and monocyte procoagulant activity, and promotes monocyte migration. *J Exp Med*. 1990; 172(6):1535–1545. [PubMed: 2258694]
141. Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marmé D. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the receptor Flt-1. *Blood*. 1996; 87(8):3336–3343. [PubMed: 8605350]
142. Celletti FL, Waugh JM, Amabile PG, Brendolan A, Hilfiker PR, Dake MD. Vascular endothelial growth factor enhances atherosclerotic plaque progression. *Nat Med*. 2001; 7(4):425–429. [PubMed: 11283668]
143. Lopez JJ, Laham RJ, Stamler A, et al. VEGF administration in chronic myocardial ischemia in pigs. *Cardiovasc Res*. 1998; 40(2):272–281. [PubMed: 9893720]
144. Lazarous DF, Shou M, Scheinowitz M, et al. Comparative effects of basic fibroblast growth factor and vascular endothelial growth factor on coronary collateral development and the arterial response to injury. *Circulation*. 1996; 94(5):1074–1082. [PubMed: 8790049]

145. Pérez-López FR, Chedraui P, Haya J, Cuadros JL. Effects of the Mediterranean diet pattern on longevity and age-prevalent morbid conditions. *Maturitas*. 2009; 64(2):67–79. [PubMed: 19720479]
146. Oak MH, Chataigneau M, Keravis T, et al. Red wine polyphenolic compounds inhibit vascular endothelial growth factor expression in vascular smooth muscle cells by preventing the activation of the p38 mitogen-activated protein kinase pathway. *Arterioscler Thromb Vasc Biol*. 2003; 23(6):1001–1007. [PubMed: 12676803]
147. Chan PS, Cervoni P. Prostaglandins, prostacyclin, and thromboxane in cardiovascular diseases. *Drug Dev Res*. 1986; 7(4):341–359.
148. Moncada S, Higgs EA. Prostaglandins in the pathogenesis and prevention of vascular disease. *Blood Rev*. 1987; 1(2):141–145. [PubMed: 3332095]
149. Iñiguez MA, Cacheiro-Llaguno C, Cuesta N, Díaz-Muñoz MD, Fresno M. Prostanoid function and cardiovascular disease. *Arch Physiol Biochem*. 2008; 114(3):201–209. [PubMed: 18629685]
150. Gleim S, Kasza Z, Martin K, Hwa J. Prostacyclin receptor/thromboxane receptor interactions and cellular responses in human atherothrombotic disease. *Curr Atheroscler Rep*. 2009; 11(3):227–235. [PubMed: 19361355]
151. Fetalvero KM, Shyu M, Nomikos AP, et al. The prostacyclin receptor induces human vascular smooth muscle cell differentiation via the protein kinase A pathway. *Am J Physiol Heart Circ Physiol*. 2006; 290(4):H1337–H1346. [PubMed: 16399867]
152. Arehart E, Gleim S, Kasza Z, Fetalvero KM, Martin KA, Hwa J. Prostacyclin, atherothrombosis, and cardiovascular disease. *Curr Med Chem*. 2007; 14(20):2161–2169. [PubMed: 17691954]
153. Chataigneau T, Feletou M, Huang PL, Fishman MC, Duhault J, Vanhoutte PM. Acetylcholine-induced relaxation in blood vessels from endothelial nitric oxide synthase knockout mice. *Br J Pharmacol*. 1999; 126(1):219–226. [PubMed: 10051139]
154. Sugatani J, Miwa M, Komiyama Y, Ito S. High-density lipoprotein inhibits the synthesis of platelet-activating factor in human vascular endothelial cells. *J Lipid Mediat Cell Signal*. 1996; 13(1):73–88. [PubMed: 8998599]
155. Naqvi TZ, Shah PK, Ivey PA, et al. Evidence that high-density lipoprotein cholesterol is an independent predictor of acute platelet-dependent thrombus formation. *Am J Cardiol*. 1999; 84(9):1011–1017. [PubMed: 10569655]
156. Sellers MM, Stallone JN. Sympathy for the devil: the role of thromboxane in the regulation of vascular tone and blood pressure. *Am J Physiol Heart Circ Physiol*. 2008; 294(5):H1978–H1986. [PubMed: 18310512]
157. Davidge ST. Prostaglandin H synthase and vascular function. *Circ Res*. 2001; 89(8):650–660. [PubMed: 11597987]
158. Hong BK, Kwon HM, Lee BK, et al. Coexpression of cyclooxygenase-2 and matrix metalloproteinases in human aortic atherosclerotic lesions. *Yonsei Med J*. 2000; 41(1):82–88. [PubMed: 10731924]
159. Tamagaki T, Sawada S, Imamura H, et al. Effects of high-density lipoproteins on intracellular pH and proliferation of human vascular endothelial cells. *Atherosclerosis*. 1996; 123(1-2):73–82. [PubMed: 8782838]
160. Cockerill GW, Saklatvala J, Ridley SJ, et al. High-density lipoproteins differentially modulate cytokine-induced expression of E-selectin and cyclooxygenase-2. *Arterioscler Thromb Vasc Biol*. 1999; 19(4):910–917. [PubMed: 10195917]
161. Herr D, Rodewald M, Fraser HM, et al. Regulation of endothelial proliferation by the renin-angiotensin system in human umbilical vein endothelial cells. *Reproduction*. 2008; 136(1):125–130. [PubMed: 18390690]
162. Shi RZ, Wang JC, Huang SH, Wang XJ, Li QP. Angiotensin II induces vascular endothelial growth factor synthesis in mesenchymal stem cells. *Exp Cell Res*. 2009; 315(1):10–15. [PubMed: 18973754]
163. Ide N, Hirase T, Nishimoto-Hazuku A, Ikeda Y, Node K. Angiotensin II increases expression of IP-10 and the renin-angiotensin system in endothelial cells. *Hypertens Res*. 2008; 31(6):1257–1267. [PubMed: 18716376]

164. Celi A, Del Fiorentino A, Cianchetti S, Pedrinelli R. Tissue factor modulation by angiotensin II: a clue to a better understanding of the cardiovascular effects of renin-angiotensin system blockade? *Endocr Metab Immune Disord Drug Targets*. 2008; 8(4):308–313. [PubMed: 19075785]
165. Shan H, Bai X, Chen X. Angiotensin II induces endothelial cell senescence via the activation of mitogen-activated protein kinases. *Cell Biochem Funct*. 2008; 26(4):459–466. [PubMed: 18383564]
166. Vinh A, Widdop RE, Drummond GR, Gaspari TA. Chronic angiotensin IV treatment reverses endothelial dysfunction in ApoE-deficient mice. *Cardiovasc Res*. 2008; 77(1):178–187. [PubMed: 18006471]
167. Sluimer JC, Gasc JM, Hamming I, et al. Angiotensin-converting enzyme 2 (ACE2) expression and activity in human carotid atherosclerotic lesions. *J Pathol*. 2008; 215(3):273–279. [PubMed: 18498093]
168. Van Linthout S, Spillmann F, Lorenz M, et al. Vascular-protective effects of high-density lipoprotein include the downregulation of the angiotensin II type 1 receptor. *Hypertension*. 2009; 53(4):682–687. [PubMed: 19273745]
169. Lu H, Rateri DL, Feldman DL, et al. Renin inhibition reduces hypercholesterolemia-induced atherosclerosis in mice. *J Clin Invest*. 2008; 118(3):984–993. [PubMed: 18274671]
170. Javeshghani D, Sairam MR, Neves MF, Schiffrin EL, Touyz RM. Angiotensin II induces vascular dysfunction without exacerbating blood pressure elevation in a mouse model of menopause-associated hypertension. *J Hypertens*. 2006; 24(7):1365–1373. [PubMed: 16794486]
171. Xu X, Xiao JC, Luo LF, et al. Effects of ovariectomy and 17beta-estradiol treatment on the renin-angiotensin system, blood pressure, and endothelial ultrastructure. *Int J Cardiol*. 2008; 130(2):196–204. [PubMed: 18083251]
172. Harvey PJ, Morris BL, Su W, Notarius CF, Miller JA, Floras JS. Estrogen replacement in postmenopausal women activates the renin-angiotensin system at rest and during simulated orthostatic stress but lowers blood pressure. *Am J Hypertens*. 2003; 16(S1):260A–261A.
173. Ichikawa J, Sumino H, Ichikawa S, Ozaki M. Different effects of transdermal and oral hormone replacement therapy on the renin-angiotensin system, plasma bradykinin level, and blood pressure of normotensive postmenopausal women. *Am J Hypertens*. 2006; 19(7):744–749. [PubMed: 16814131]
174. Umeda M, Ichikawa S, Kanda T, Sumino H, Kobayashi I. Hormone replacement therapy increases plasma level of angiotensin II in postmenopausal hypertensive women. *Am J Hypertens*. 2001; 14(3):206–211. [PubMed: 11281230]
175. Langrish JP, Mills NL, Bath LE, et al. Cardiovascular effects of physiological and standard sex steroid replacement regimens in premature ovarian failure. *Hypertension*. 2009; 53(5):805–811. [PubMed: 19332659]
176. Kalantaridou SN, Naka KK, Papanikolaou E, et al. Impaired endothelial function in young women with premature ovarian failure: normalization with hormone therapy. *J Clin Endocrinol Metab*. 2004; 89(8):3907–3913. [PubMed: 15292326]
177. Connell JM, MacKenzie SM, Freel EM, Fraser R, Davies E. A lifetime of aldosterone excess: long-term consequences of altered regulation of aldosterone production for cardiovascular function. *Endocr Rev*. 2008; 29(2):133–154. [PubMed: 18292466]
178. Perez-Lopez FR. Clinical experiences with drospirenone: from reproductive to postmenopausal years. *Maturitas*. 2008; 60(2):78–91. [PubMed: 18468818]
179. Wang M, Zukas AM, Hui Y, Ricciotti E, Puré E, FitzGerald GA. Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis. *Proc Natl Acad Sci U S A*. 2006; 103(39):14507–14512. [PubMed: 16973753]
180. Pérez-López FR. Vitamin D and its implications for musculoskeletal health in women: an update. *Maturitas*. 2007; 58(2):117–137. [PubMed: 17604580]
181. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med*. 2008; 168(11):1174–1180. [PubMed: 18541825]
182. Pilz S, Dobnig H, Nijpels G, et al. Vitamin D and mortality in older men and women. *Clin Endocrinol (Oxf)*. 2009; 71(5):666–672. [PubMed: 19226272]

183. Yamamoto T, Kozawa O, Tanabe K, et al. 1,25-dihydroxyvitamin D3 stimulates vascular endothelial growth factor release in aortic smooth muscle cells: role of p38 mitogen-activated protein kinase. *Arch Biochem Biophys.* 2002; 398(1):1–6. [PubMed: 11811942]
184. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr.* 2006; 83(4):754–759. [PubMed: 16600924]
185. Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med.* 2008; 168(12):1340–1349. [PubMed: 18574092]
186. Yang R, Barouch LA. Leptin signaling and obesity. Cardiovascular consequences. *Circulation Res.* 2007; 101(6):545–549. [PubMed: 17872473]
187. Kralisch S, Bluher M, Paschke R, Stumvoll M, Fasshauer M. Adipokines and adipocyte targets in the future management of obesity and the metabolic syndrome. *Mini Rev Med Chem.* 2007; 7(1):39–45. [PubMed: 17266636]
188. Halberg N, Wernstedt-Asterholm I, Scherer PE. The adipocyte as an endocrine cell. *Endocrinol Metab Clin North Am.* 2008; 37(3):753–768. [PubMed: 18775362]
189. Matarese G, Mantzoros C, La Cava A. Leptin and adipocytokines: bridging the gap between immunity and atherosclerosis. *Curr Pharm Des.* 2007; 13(36):3676–3680. [PubMed: 18220805]
190. Rahmouni K, Haynes WG. Leptin and the cardiovascular system. *Recent Prog Horm Res.* 2004; 59:225–244. [PubMed: 14749504]
191. Fried SK, Ricci MR, Russell CD, LaFerrere B. Regulation of leptin production in humans. *J Nutr.* 2000; 130(12):3127S–3131S. [PubMed: 11110887]
192. Wallace AM, McMahon AD, Packard CJ, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation.* 2001; 104(25):3052–3056. [PubMed: 11748099]
193. Wolk R, Berger P, Lennon RJ, Brilakis ES, Johnson BD, Somers VK. Plasma leptin and prognosis in patients with established coronary atherosclerosis. *J Am Coll Cardiol.* 2004; 44(9):1819–1824. [PubMed: 15519013]
194. Shek EW, Brands MW, Hall JE. Chronic leptin infusion increases arterial pressure. *Hypertension.* 1998; 31(1 pt 2):409–414. [PubMed: 9453337]
195. Correia ML, Morgan DA, Sivitz WI, Mark AL, Haynes WG. Leptin acts in the central nervous system to produce dose-dependent changes in arterial pressure. *Hypertension.* 2001; 37(3):936–942. [PubMed: 11244021]
196. Sierra-Honigsmann MR, Nath AK, Murakami C, et al. Biological action of leptin as an angiogenic factor. *Science.* 1998; 28(5383):1683–1686. [PubMed: 9733517]
197. Fruhbeck G. Pivotal role of nitric oxide in the control of blood pressure after leptin administration. *Diabetes.* 1999; 48(4):903–908. [PubMed: 10102710]
198. Lembo G, Vecchione C, Fratta L, et al. Leptin induces direct vasodilation through distinct endothelial mechanisms. *Diabetes.* 2000; 49(2):293–297. [PubMed: 10868946]
199. Kimura K, Tsuda K, Baba A, et al. Involvement of nitric oxide in endothelium-dependent arterial relaxation by leptin. *Biochem Biophys Res Commun.* 2000; 273(2):745–749. [PubMed: 10873674]
200. Gardiner SM, Kemp PA, March JE, Bennett T. Regional haemodynamic effects of recombinant murine or human leptin in conscious rats. *Br J Pharmacol.* 1999; 130(4):805–810. [PubMed: 10864886]
201. Korda M, Kubant R, Patton S, Malinski T. Leptin-induced endothelial dysfunction in obesity. *Am J Physiol Heart Circ Physiol.* 2008; 295(4):H1514–H1521. [PubMed: 18689498]
202. Brydon L, O'Donnell K, Wright CE, Wawrzyniak AJ, Wardle J, Steptoe A. Circulating leptin and stress-induced cardiovascular activity in humans. *Obesity (Silver Spring).* 2008; 16(12):2642–2647. [PubMed: 18820649]
203. Beltowski J, Jamroz-Wiśniewska A, Widomska S. Adiponectin and its role in cardiovascular diseases. *Cardiovasc Hematol Disord Drug Targets.* 2008; 8(1):7–46. [PubMed: 18336252]

204. Okamoto Y, Arita Y, Nishida M, et al. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. *Horm Metab Res.* 2000; 32(2):47–50. [PubMed: 10741683]
205. Yamauchi T, Kamon J, Ito Y, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature.* 2003; 423(6941):762–769. [PubMed: 12802337]
206. Bråkenhielm E, Veitonmäki N, Cao R, et al. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. *Proc Natl Acad Sci U S A.* 2004; 101(8):2476–2481. [PubMed: 14983034]
207. Ukkola O, Santaniemi M. Adiponectin: a link between excess adiposity and associated comorbidities? *J Mol Med.* 2002; 80(11):696–702. [PubMed: 12436346]
208. Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med.* 2002; 8(7):731–737. [PubMed: 12068289]
209. Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. *Circulation.* 1999; 99(13):1726–1732. [PubMed: 10190883]
210. Kizer J, Barzilay JI, Kuller LH, Gottdiener JS. Adiponectin and risk of coronary heart disease in older men and women. *J Clin Endocrin Metab.* 2008; 93(9):3357–3364.
211. Chen BH, Song Y, Ding EL, et al. Circulating levels of resistin and risk of type 2 diabetes in men and women: results from two prospective cohorts. *Diabetes Care.* 2009; 32(2):329–334. [PubMed: 18957529]
212. Butler J, Kalogeropoulos A, Georgiopoulou V, et al. for the Health ABC Study. Serum resistin concentrations and risk of new onset heart failure in older persons: the health, aging, and body composition (Health ABC) study. *Arterioscler Thromb Vasc Biol.* 2009; 29(7):1144–1149. [PubMed: 19372460]
213. Frankel DS, Vasani RS, D'Agostino RB Sr, et al. Resistin, adiponectin, and risk of heart failure the Framingham offspring study. *J Am Coll Cardiol.* 2009; 53(9):754–762. [PubMed: 19245965]
214. Papadopoulos DP, Perrea D, Thomopoulos C, et al. Masked hypertension and atherogenesis: the impact on adiponectin and resistin plasma levels. *J Clin Hypertens (Greenwich).* 2009; 11(2):61–65. [PubMed: 19222669]
215. Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BL, Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *Eur J Endocrinol.* 2003; 149(4):331–335. [PubMed: 14514348]
216. Vendrell J, Broch M, Vilarrasa N, et al. Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. *Obes Res.* 2004; 12(6):962–971. [PubMed: 15229336]
217. Lewandowski KC, Szosland K, O'Callaghan C, Tan BK, Randeve HS, Lewinski A. Adiponectin and resistin serum levels in women with polycystic ovary syndrome during oral glucose tolerance test: a significant reciprocal correlation between adiponectin and resistin independent of insulin resistance indices. *Mol Genet Metab.* 2005; 85(1):61–69. [PubMed: 15862282]
218. Travers S, Jeffers B, Bloch C, Hill J, Eckel R. Gender and Tanner stage differences in body composition and insulin sensitivity in early pubertal children. *J Clin Endocrinol Metab.* 1995; 80(1):172–178.
219. Moran A, Jacobs D, Steinberger J, et al. Insulin resistance during puberty: results from clamp studies. *Diabetes.* 1999; 48(10):2039–2044. [PubMed: 10512371]
220. Wilkin T, Murphy M. The gender insulin hypothesis. *Int J Obes.* 2006; 30(7):1056–1061.
221. Pan W, Cedres L, Liu K. Relationship of clinical diabetes and asymptomatic hyperglycemia to risk of coronary heart disease mortality in men and women. *Am J Epidemiol.* 1986; 123(3):504–516. [PubMed: 3946397]
222. Lundberg V, Stegmayr B, Asplund K. Diabetes as a risk factor for myocardial infarction: population and gender perspectives. *J Intern Med.* 1997; 241(6):485–492. [PubMed: 10497624]
223. Regitz-Zagrosek V, Lehmkuhl E, Mahmoodzadeh S. Gender aspects of the role of the metabolic syndrome as a risk factor for cardiovascular disease. *Gend Med.* 2007; 7(2):130–139.
224. Abernethy T, Francis T. Studies on the somatic c polysaccharide of pneumococcus. *J Exp Med.* 1937; 65(1):59–73. [PubMed: 19870591]

225. Manolakou P, Angelopoulou R, Bakoyiannis C. The effects of endogenous and exogenous androgens on cardiovascular disease risk factors and progression. *Reprod Biol Endocrinol*. 2009; 12(7):44. [PubMed: 19433001]
226. Sullivan J. Iron and the sex difference in heart disease risk. *Lancet*. 1981; 1(8233):1293–1294. [PubMed: 6112609]
227. Heinecke J. Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. *Atherosclerosis*. 1998; 141(1):1–15. [PubMed: 9863534]
228. Friedrich N, Milman N, Volzke H, Linneberg A, Jorgensen T. Is serum ferritin within the reference range a risk predictor of cardiovascular disease? *Br J Nutr*. 2009; 102(4):594–600. [PubMed: 19203421]
229. Van Der A, Grobbee D, Roest M. Serum ferritin is a risk factor for stroke in postmenopausal women. *Stroke*. 2003; 36(8):1637–1641.
230. Sainani G, Sainani R. Homocysteine and its role in the pathogenesis of atherosclerotic vascular disease. *J Assoc Physicians India*. 2002; 50(suppl 1):16–23. [PubMed: 12186151]
231. Selhub J. Homocysteine metabolism. *Annu Rev Nutr*. 1999; 19:217–246. [PubMed: 10448523]
232. Wilson K, Lentz S. Mechanisms of the atherogenic effects of elevated homocysteine in experimental models. *Semin Vasc Med*. 2005; 5(2):163–171. [PubMed: 16047268]
233. Bona K, Njolstad I, Ueland P. NORVIT trial investigators. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med*. 2006; 354(15):1578–1588. [PubMed: 16531614]
234. Papatheodorou L, Weiss N. Vascular oxidant stress and inflammation in hyperhomocysteinemia. *Antioxid Redox Signal*. 2007; 9(11):1941–1958. [PubMed: 17822365]
235. Domagala T, Undas A, Libura M, Szczeklik A. Pathogenesis of vascular disease in hyperhomocysteinemia. *J Cardiovasc Risk*. 1998; 5(4):239–247. [PubMed: 9919472]
236. Coppola A, Davi G, DeStefano V, Mancini F, Cerbone A, DiMinno G. Homocysteine, coagulation, platelet function, and thrombosis. *Semin Thromb Hemost*. 2000; 26(3):243–254. [PubMed: 11011842]
237. Zhi Y, Huang Y, Li Z, Zhang R. Hypermethylation of estrogen receptor-alpha gene and high homocysteine in atheromatosis patients. *Wei Sheng Yan Jiu*. 2008; 37(3):314–317. [PubMed: 18646531]
238. Bonassi Machado R, Baracat EC, Fernandes CE, Lakryc EM, Rodrigues De Lima G. Effects of estrogen and estrogen/progestogen therapy on homocysteine levels and their correlation with carotid vascular resistance. *Gynecol Endocrinol*. 2007; 23(11):619–624. [PubMed: 17907004]
239. Nakhai Pour HR, Grobbee DE, Muller M, Emmelot-Vonk M, van der Schouw YT. Serum sex hormone and plasma homocysteine levels in middle-aged and elderly men. *Eur J Endocrinol*. 2006; 155(6):887–893. [PubMed: 17132759]