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Sex Differences in Cardiovascular Disease with Implications for Therapies

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Abstract

A consortium of investigator-thought leaders was convened at the Heart Institute at Cedars-Sinai Medical Center and produced the following summary points:

- **Point 1** - Important sex differences exist in cardiovascular disease (CVD) which impact disease initiation, diagnosis and treatment.
- **Implication** – Research that acknowledges these differences is needed to optimize outcomes in women and men.

- **Point 2** - Atherosclerosis is qualitatively and quantitatively different in women and men; women demonstrate more plaque erosion, more diffuse plaque with less focal artery lumen intrusion.
- **Implication** – CVD strategies that include devices should explore differing anatomical shapes and surfaces as well differing drug coating and eluting strategies.

- **Point 3 -** Bone marrow progenitor cells (PCs) engraft differently based on the sex of the donor cell and the sex of the recipient.
- **Implication** – PC therapeutic studies need to consider the sex of cells of the source and the recipient.

- **Point 4** – Women have a greater risk of venous but not arterial thrombosis compared to men as well as more bleeding complications related to anticoagulant

treatment. Several genes coding for proteins involved in hemostasis are regulated by sex hormones.

- **Implications** – Thrombosis management with CVD strategies that include devices should be preferentially tested in women.

- **Point 5** – Women and men can have differences in pharmacological response.
- **Implication** – Sex-specific pharmacogenomics should be included in pharmacological development.

- **Point 6** – CVD progression results from an imbalance of cell injury and repair due to insufficient PC repair, which is impacted by sex differences.
- **Implication** – CVD regenerative strategies should be directed at learning to deliver cells that shift the recipient balance from injury toward repair.

- **Point 7** – Females have higher circulating levels of PCs with greater migratory capacity and higher rates of tissue repair.
- **Implication** – CVD tissue repair strategies should ideally be tested first in females to have the best chance of success for proof-of-concept, followed by testing in the more challenging male models.

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Introduction. ***Drs. Saralyn Mark and C. Noel Bairey Merz***

Cardiovascular (CVD) is the leading killer of both women and men. Sex differences in CVD outcomes exist, and yet knowledge of sex differences in CVD, ranging from basic science inquiry to pathophysiological understanding, diagnostics and therapeutics, is limited. A symposium of thought leaders was convened at Cedars-Sinai Medical Center to address this issue with the following three objectives: 1) to determine the current knowledge of sex differences in CVD; 2) to identify important knowledge gaps in sex differences in CVD and prioritize research areas; and 3) to identify challenges and barriers to the inclusion of sex differences investigation in CVD.

The Institute of Medicine (IOM) in 2001 published a monograph exploring biological contributions to human health entitled "Does Sex Matter?"[1]. The IOM report came up with three conclusions: 1) sex does matter, and should be considered when designing and analyzing studies in all areas of health-related research; 2) the study of sex differences has been predominantly observational research, and the next step is study of mechanisms and therapies related to sex differences when present; and 3) barriers to the advancement of knowledge about sex differences in health and illness must be eliminated.

To understand sex differences research, a discussion of terminology is needed. The term "sex" refers to the biological sexual differentiation (e.g., women have ovaries and men have testes). The term "gender" refers to the socio-cultural attributes of that biological sex (e.g., women have complex social networks and men have wives). The term "sex genotype" refers to the XX and XY chromosomes. "Sex phenotype" refers to the phenotypic expression of the genotype (e.g., ovarian development in females and

testicular development in males). Phenotypic expression may vary with development and age (e.g., premenopausal women have higher estrogen levels due to ovulation compared to post- menopausal women who do not ovulate). Both remain XX genotype but differ in phenotypic expression.

Examples of sex differences are evident in a wide variety of biomedical areas. Women have greater high frequency non-auditory brain wave patterns when tested compared to men, whereas homosexual men are intermediate between women and heterosexual men, suggesting that this may be genotypic[2]. Women have lower thermal pain thresholds than men which appear to be mediated by estrogen levels suggesting that this sex difference is phenotypic[3]. Women are evaluated less often than men by physicians in response to chest discomfort, suggesting cultural sexism bias[4].

This symposium is timely because women do not appear to be benefiting optimally from current CVD strategies. While overall CVD has declined by 52% in men and 49% in women, more women now die annually from CVD than men, and there is an actual *increase* in mortality in younger middle-aged women[5]. Thought leaders and representatives from the Organization for the Study of Sex Differences (OSSD), the American College of Cardiology (ACC), the American Heart Association (AHA), and the National Heart, Lung and Blood Institute (NHLBI) participated. The symposium attendees are listed in the Appendix. The symposium summary points are presented in Table 1.

Sex Differences in CVD Prevalence and Risk Factors: *Dr. Leslee J. Shaw*

There is a delay in the onset of CVD in women that is generally about 10 years beyond that of their male counterparts[6]. Hypoestrogenemia and hyperandrogen states can

alter the prevalence and clustering of cardiac risk factors as well as result in an heightened risk of CVD at a younger age [7-9]. Attention to sex-specific issues related to CVD has contributed to recent declines in disease-specific mortality; as reported from the CDC's National Center for Health Statistics noting 10,000 fewer annual deaths in women since 2000 (a 10% reduction)(Figure 1)[10].

Sex-based differences in the prevalence, clustering, and outcomes of cardiac risk factors supports a unique profile that accentuates long-term risk. For women, both hypertriglyceridemia and diabetes are associated with relatively worse CVD outcomes when compared to men[6]. CVD risk factors increase at the time of menopause, often concomitant with weight gain. Post-menopause, a mild decline in HDL cholesterol, while age-related arterial stiffness contributes to the rising prevalence of hypertension.

C-reactive protein is a novel inflammatory marker and is also more often elevated in women as compared to men[11], and may contribute to this differing plaque pathophysiology[12]. The WISE investigators have hypothesized that the combination of prolonged exposure to atherogenic risk factors, sex-specific inflammatory status that includes autoimmune disease, reproductive hormonal status, positive arterial remodeling and microvascular dysfunction leads to a more complicated course for women with established CVD compared to men [6, 13, 14].

Reproductive hormones also appear to be involved. Low estrogen levels premenopausally due dysruption of ovulatory cycling are associated with greater obstructive CAD[7]. We have also evaluated hormonal alterations associated with polycystic ovary syndrome (PCOS), which is a risk factor for type-2 diabetes mellitus and the metabolic syndrome that includes hyperlipidemia, central obesity, and insulin

resistance. Women who fit a PCOS phenotype that includes elevated androgen levels have elevated CVD risk in the post-menopause[8]. This work suggests that imbalance in endogenous sex hormones in women accelerates CVD risk.

Sex differences in direct markers of subclinical atherosclerosis, such as coronary artery calcification (CAC), have also been reported[15, 16]. In a report on mortality differences by sex in 10,377 asymptomatic individuals referred for evaluation of CVD risk[16], a higher mortality existed risk in the setting of CAC for women compared to men. In a related report, Bellasi and colleagues noted a higher mortality risk for women with CAC who had 3 or more risk factors[15, 16] Both report support the degree to which comorbidity and clustered risk factors potentiate the atherosclerotic disease process and heighten risk in women as compared to men.

Among symptomatic patients, women have less obstructive angiographic coronary artery disease (CAD) compared to men. In a recent multi-national analysis, the prevalence of angina is greater in women as compared to men up to age 75 years[17]; supporting prior reports that CVD presentation and risk varies by age. In a report from a nation-wide registry of 835,827 patients with chest pain, including 459,941 acute coronary syndrome (ACS) patients, despite the less frequent obstructive CAD[18], in-hospital mortality for stable chest pain and ACS patients was consistently higher for women compared to men[19]. In a recent pooled analysis of data from several ACS trials, those at highest risk included those with mild coronary disease compared to completely normal arteries[20]; younger women in particular had higher hospital mortality when compared to younger men, despite less obstructive CAD[21]. Many women with non-obstructive CAD have persistent symptoms with nearly half complaining of chest pain

through 5 years of follow-up[22] and nearly 1 in 5 requires hospitalization for refractory or worsening angina. Evidence of ischemia accelerates risk for symptomatic women of all ages[23], and symptoms with even mild CAD elevate risk in women[24]. The limited understanding of the mechanisms sex differences in CVD places women at elevated risk[25].

Sex Differences in Atherosclerosis and Response to Intimal Injury. *Dr. Prediman K. Shah*

Glagov et al[26] observed that arteries undergo positive remodeling by enlarging outward in order to accommodate plaque and avoid luminal compromise. Proteases produced by inflammatory cells appear to have a role in adventitial remodeling through extracellular matrix destruction[27], suggesting that the inflammatory processes are relevant in outward remodeling. Women appear to have more diffuse atherosclerosis, less luminal stenosis, higher incidence of endothelial dysfunction, and a higher prevalence of microvascular dysfunction compared to men[19 19], suggesting that women may have greater positive remodeling.

The patho-anatomic substrate for coronary thrombosis also differs between men and women. In men, 80% of thrombi tend to occur due to plaque rupture, whereas in women 20-40% of the coronary thrombi occur on an intact atherosclerotic plaque with superficial athero-intimal erosion (Figure 2) [12 28]. This plaque erosion is a common finding in SCD in younger women, who were smokers and post-menopausal women on estrogens. Conversely, plaque rupture leading to thrombosis is relatively more common in men and older women. .

Plaques that tend to rupture are composed of a large lipid core laden atherosclerotic lesion with intimal and adventitial inflammation and increased plaque neovascularity[27]. Inflammatory cells trigger death of smooth muscle cells through apoptosis and produce matrix-degrading enzymes which can induce depletion of the collagen framework leading to loss of collagen and thinning of the fibrous cap[27] and eventually either rupture. Importantly, lipid-filled plaques have inflammatory cell-derived tissue factor (TF) that is a prototypical trigger for activating the clotting cascade. When a lipid-rich plaque ruptures, TF is immediately exposed to circulating blood, which with other factors, stimulates the production of thrombin, which in turn leads to platelet-fibrin thrombus formation[27].

The mechanisms of sex differences in this process are not well understood. Enhanced endothelial apoptosis is associated with exposure of TF on the luminal side, and a higher prevalence of superficial endothelial erosions with increased sex-specific circulating coagulability[29, 30]. Also systemic inflammatory processes increase anti-cardiolipin antibodies, which are more prevalent in women, and TF which may not be originating from the plaque but from the circulation[31] may also create a prothrombatic state. Normally TF remains contained within circulating leucocytes, and is not available to trigger thrombosis. However under certain conditions, circulating leucocytes can shed membrane micro-particles, which by electron microscopy have been shown to be laden with TF [31]. Accordingly, these micro-particles can be delivered to platelets and to other circulating leukocytes, and the transfer of TF from circulating cells can occur at the site of endothelial erosion. This can cause thrombosis even though the plaque does not contribute TF.

Spontaneous coronary artery dissection (SCAD) is a rare clinical syndrome that is more prevalent in women compared to men. In fact 80% of cases occur in women, particularly in pre-menopausal women, often in the peri-partum setting[32]. The clinical presentation frequently is SCD and less commonly unstable angina, acute MI, heart failure or shock. Interestingly, the left anterior descending is more commonly affected in women, whereas the right coronary is more commonly affected in men, and simultaneous multiple vessel dissections can also occur. Reproductive hormones may contribute to this, in that matrix metalloproteinases may be induced by hormonal alterations and may promote intimal disruption and dissection.

The Tako-Tsubo Syndrome, also known as stress-induced cardiomyopathy[33, 34], is also more prevalent in women, especially in older post-menopausal women. These patients present with findings of an ACS, often triggered by positive or negative emotional or surgical stress. Patients can present in shock, and ventricular function assessment frequently shows ballooning of the left ventricular (LV) apex and preserved or vigorous contraction of the base with no obstructive CAD, low to modest cardiac enzyme elevation and LV recovery in 4-8 weeks [33, 34]. Previously considered a rare syndrome, it appears to account for 2-5% of patients presenting with clinical picture of an ACS[33]. The pathophysiology of the Tako-Tsubo Syndrome has been hypothesized to be an outcome of: 1) transient LV stunning induced by a sudden surge of catecholamines; 2) coronary spasm and/or microvascular dysfunction; and 3) coronary thrombosis followed by rapid spontaneous recanalization. Furthermore, it has more recently been hypothesized that Tako-Tsubo syndrome represents a form of myocardial stunning that differs mechanistically from post-ischemic stunning[35].

Inspection of the mechanisms involved in atherosclerosis and intimal response to injury, including arterial remodeling, plaque composition, destabilization and thrombosis, as well as arterial dissection and microvascular dysfunction contribute to understanding of the observed sex differences in CVD. Atherosclerosis is qualitatively and quantitatively different in women and men; women demonstrate more plaque erosion, more diffuse plaque with less focal artery lumen intrusion. Investigation into these areas aimed at more fully understanding sex-specific mechanisms should be targeted in order to develop tailored therapies.

Sex Difference in Bone and Vascular Cellular and Tissue Engineering. ***Dr. Barbara D. Boyan***

The study of bone tissue provides an example of sex based-differences in mechanism for CVD investigators to emulate. Sex differences in properties of bone are established in utero, due to sex traits that are inherited differently in males and in females, and relate to vascular cellular and tissue engineering. A family of proteins called *small integrin-binding N-linked glycoprotein* (SIBLING) that regulate cell migration and cell attachment are encoded on the X chromosome, such that when there is a genetic change in one of these molecules, it is experienced to a greater extent in males[36]. Studies examining the response of male and female cartilage cells to estrogen show that while both sexes have receptors for estrogen, some of the estrogen actions are seen only in females[37]. In contrast, male cartilage cells exhibit responses to testosterone that are not observed in female cells[38]. Cartilage cells and bone cells make their own estrogen and their own testosterone, although it's regulated differently in the two cell types[39, 40].

Huard et al, have demonstrated that muscle-derived, mesenchymal stem cells (MSCs) from females engraft easily into female recipients, and less well into males. Male cells were even less effective in engrafting into females, and the least effective engraftment of muscle-derived MSCs was male cells into males[41]. These results suggest important sex differences in stem cells that are more than a simple male-female difference.

Bone is a tissue that is constantly remodeling including its vascular blood supply. Studies indicate that osteoblasts are sensitive to the physical and chemical properties of the bone surface[42]. We have taken advantage of a cell culture model in which osteoblasts are grown on well controlled titanium (Ti) substrates with specific microstructural elements. In response to estradiol, female cells produce large amounts of TGF-beta on that surface, compared to male cells [43], which do not respond to estrogen in this particular outcome (Olivares-Navarrete et al., unpublished data). This has potential important implications for attempts to resurface vascular stents, where endothelial response to the material surface and subsequent re-endothelialization is also a major concern.

Studies using ablation of a rat tibial bone marrow model demonstrates initiation of a sequence of events that include formation of a hematoma within the marrow cavity (days 1-3), stimulation of rapid bone formation throughout the marrow cavity with onset of mineralization at day 6, osteoclast resorption of the newly formed bone at day 21, and restoration of marrow by day 28. Results from these studies show that when biomaterials are implanted in the marrow cavity, endosteal bone formation is affected, the restoration of the marrow is affected, and a material-specific systemic response occurs as well. Interestingly, distribution of the vascular imaging agent Tc99-MDP is also affected by

the presence of the biomaterial[44]. Growth of cells on Ti substrates with different microstructures and chemistry affects the release of angiogenic factors including vascular endothelial growth factor-A (VEGF-A), endothelial growth factor (EGF) and fibroblast growth factor-2 (FGF-2) (Raines et al., unpublished data). Surfaces that are highly effective in promoting bone formation are also more vasculogenic, and that this correlates with the differentiation of the osteoblasts. Accordingly, the growth factor "cocktail" that is produced by these cells on the highly osteogenic surface is in fact also highly vasculogenic. Whether this is also sex-specific is of great interest, particularly when using materials as implants in osteoporotic bone[45], or to ensure new blood vessel growth for tissue vascular supply[46].

Understanding sex differences in tissue development could have widespread implications for a variety of CVD therapies, with relevance to local micro-materials and tissue vascular supply important to device development. CVD strategies that include devices should explore differing anatomical shapes and surfaces as well differing drug coating and eluting strategies.

Progenitor Cell and Cytokine Regulation of Cardiovascular Tissue: Implications for Repair/Restenosis. ***Dr. Doris Taylor***

Cell-based cardiovascular repair is not only feasible but likely because virtually every organ in the body either contains tissue-specific progenitor cells (PCs) or is linked to a reservoir of cells in bone marrow that are mobilized and migrate into tissues when needed[47-55]. We also now know that chronic disease and aging is associated with a reduced number and functional capacity of PCs[56-60]. We propose that a major role of inflammation in the setting of chronic disease or acute injury is to recruit "appropriate"

reparative PCs to the injury site and start an endogenous repair process. Further, if appropriate cells reach the site of injury and either deliver a small molecule (e.g., cytokine) or physically integrate, the inflammatory response decreases[51-55]. However, in the absence of sufficient cell numbers or recruitment for repair, there is a deleterious positive feedback loop, (e.g., increased inflammation) that occurs. This is characterized by a stronger pro-inflammatory T helper (TH)-1-type cytokine (i.e., TNF-alpha and interleukin [IL]-12) response, and recruitment of pro-inflammatory cells to the injury site[61].

In preclinical models, there are sex differences in this system of repair in response to atherosclerotic disease[62], including differences in the composition of bone marrow, and responses to treatment with bone marrow-derived cells, which are reflected in differences in serum cytokines and the repair milieu[57]. For example, although bone marrow-derived CD34-positive cells (associated with vasculogenesis) decrease with age in both males and females, there are sex differences in the pattern of decrease that inversely correlate with progression of atherosclerotic disease[52, 57,63]. Another cell population, vascular progenitor or CD31(+)/CD45 (low) cells, also linearly decreases with age and strongly predicts the onset of atherosclerosis[62] in females, suggesting that this may be a potential diagnostic marker in women. Finally, cells that are most often described as endothelial PCs (AC133(+)/CD34(+)) increase mildly at the onset of atherosclerosis and then decline, at which point atherosclerosis accelerates[57], suggesting an imbalance between injury and repair.

These data support the hypothesis that a cell number-mediated threshold for vascular repair exists below which repair does not occur, and that the threshold is

increased by stress, age, or ongoing disease[52]. To test this, we delivered bone marrow cells from young mice to aging animals intravenously and demonstrated a dramatic reduction in plaque burden over time in the treated animals[52] despite no effect on serum lipids. Instead, the delivered cells differentiated into endothelial cells at the level at the atherosclerotic lesion[52] and appeared to decrease plasma markers of inflammation including IL-6.

To determine if sex was also an important component of vascular repair, we gave male and female animals with severe atherosclerotic plaque four doses of bone marrow cells, one every two weeks, after which we assessed the plaque burden response[57]. Two observations were noteworthy between females and male animals. Females demonstrated a greater increase in vascular PCs and endothelial PCs in the bone marrow compared to males; and males had a greater increase in inflammatory (CD45(+)) cells compared to females. Among the treated males and females, female cell administration decreased plaque burden in males to a much greater degree than male cell administration, while female cells administration to females did not perform as well (Figure 3). Male cells administered to males or females demonstrated no improvement[62, 63] - and trended toward a worsening of plaque burden in females. Notably, the male recipients had low endogenous bone marrow endothelial PC numbers with a high plaque burden, and both male and female bone marrow cell administration increased the bone marrow endothelial PC number, but only the female cell administration decreased plaque burden. Conversely, female recipients had high endogenous bone marrow endothelial cells, and administration of bone marrow did not impact plaque burden, indicating that the mechanism of plaque deposition involves cells beyond endothelial PCs.

To gain insights into mechanism, we also quantified serum cytokines in the male and female animals and found that the administered cells carry their sex phenotype into the recipient. The greatest predictors of the atherosclerosis plaque outcome were the regulatory cytokine, IL-15 and the cell mobilizing agent, granulocyte colony-stimulating factor (GCSF).

In summary, inflammation is a key mediator of endogenous repair and a critical target to maximizing cell and gene therapy. The balance between pro- and anti-inflammatory responses differ between males and females and this is exacerbated with age. Bone marrow PCs both express pro (TH-1) and anti (TH-2)-inflammatory cytokines, and this expression differs by sex. When a pro-inflammatory response is greater than an anti-inflammatory response, endogenous PCs associated with injury are recruited.

Understanding the PC differences in women and men with regard to endogenous repair including genomic analyses should be pursued. Bone marrow progenitor cells (PCs) engraft differently based on the sex of the donor cell and the sex of the recipient. PC therapeutic studies need to consider the sex of cells of the source and the recipient.

Sex Differences in Medication Response and Pharmacogenetics. ***Dr. C. Noel Bairey Merz***

The majority of medications which are withdrawn following FDA approval are withdrawn due to unanticipated adverse effects in women[64, 65]. Women are consistently under-represented in cardiovascular clinical trial enrollment [66], resulting in an inability to document intervention efficacy in women, or to evaluate post-hoc sex differences. The NHLBI has mandated female-only studies, such as the Women's Ischemia Syndrome

Evaluation (WISE)[67], yet CVD medication and device intervention trials remain a problem. What do we know about medication response and pharmacogenetics in women: what sex differences have been described, and what gaps exist in the fund of knowledge?

Large secondary prevention studies of aspirin that included both sexes demonstrate equal efficacy in women and men for prevention of recurrent CVD[68]. Meta-analysis of the primary prevention aspirin studies that included women and men suggests no difference in response to aspirin, although insufficient numbers of women were randomized in these studies to independently test efficacy[69]. Comparison of the all-female low dose aspirin trial and the all-male Physician's Health study provides an interesting paradox that alludes to a sex difference with regard to aspirin and CVD[70]. Among women, low dose aspirin did not prevent first heart attack, yet prevented first stroke, while conversely aspirin prevented first heart attack, but not first stroke in men (Figure 4). The studies documented aspirin efficacy at the trial doses used in terms of impact on platelets, and thus it does not seem likely that this sex difference is due to differences in weight-adjusted dosing or pharmacological effect.

This observed sex difference in aspirin medication trials calls for a re-examination of observational CVD datasets for potential explanations. Observational data demonstrate that a relatively higher percentage of CVD events in women is attributable to stroke compared to a relatively higher percentage of CVD events attributable to myocardial infarction in men, suggesting that aspirin efficacy may vary according to sex differences in the pathophysiology of CVD. The size of blood vessels involved in the pathobiology of stroke (dominantly microvasculature) versus myocardial infarction (dominantly macrovasculature) suggests the hypothesis that microvascular CVD

involvement may be preferentially more important in women. Future clinical trials must sex stratify results, as well as enroll a sufficient number of women to test efficacy in both sexes.

Evaluation of secondary prevention trials of statins, cholesterol lowering medications, similarly demonstrates that statins appear to work equally effectively in women and men with established CVD[71]. The recent large primary prevention JUPITER trial, which identified subjects without evident CVD risk but with elevated hsCRP, demonstrated a robust cardiac event reduction with statin lipid-lowering therapy in women[72]. Additional clinical trials should be performed in appropriate at-risk women with this class of medication that clearly to prevent CVD which kills more young women than breast cancer [73].

Similar limitations in sex specific clinical trial data are evident with regard to beta blockers[74, 75], anti-arrhythmics such as amiodarone[76-79], and internal cardiac defibrillator (ICD) devices[80], demonstrating confidence intervals among the female subgroups that are substantially wider than those of the men due to the relatively smaller sample size resulting in insufficient statistical power to test efficacy in women. A reasonable conclusion to draw after examination of the existing datasets of clinical trials of CVD therapies in women is that of the “pedestal effect”, whereby women are neither tested nor treated because they are “special”.

Are there reasons to believe that sex differences in medication response might be real? A leading question addressed by the WISE study was to understand the pathophysiology of women with the triad of persistent symptoms, non-obstructive coronary arteries, and evidence of ischemia and/or MI by objective measures such as

stress testing or troponin levels, a condition dominantly observed in women. Indeed, women compared to men are two to three times more likely to have an ACS or MI with open coronary arteries[81], suggestive of an important sex difference. The WISE studies have demonstrated that a sizable proportion of these women, compared to a minority of men, have microvascular coronary dysfunction[82], that this correlates with persistent chest symptoms[83], and is associated with an adverse CVD prognosis[84]. Using an estimate of a 65% prevalence of persistent symptoms from the WISE and other studies[83], applied in the women enrolled in a national coronary angiography database with normal or non-obstructive coronary arteries, there are a projected 90,000 new cases annually, and as many as 2-3 million women in the US with this condition[7]. Contrasted with the 6 million women in the U.S. living with obstructive CAD, and we gain an appreciation of the magnitude of this CVD problem in women not previously recognized.

These data in women further suggest the hypothesis that sex differences in CVD pathophysiology relative to micro- and macrovascular dysfunction may contribute to our understanding of sex differences in response to medication. Review of the literature suggests that women have a greater vasoreactive state compared to men[85], and little is known regarding possible underlying genetic contributors to this sex difference. Pepine and co-investigators have demonstrated that coronary endothelial function testing in response to acetylcholine varies according to a polymorphism at the position 573 in angiotensin receptor type 1 (AT1R) in the WISE women irregardless of the presence of absence of obstructive CAD[86]. This finding supports a genetic component to coronary endothelial dysfunction in women; comparative research in men is needed to identify the putative sex difference. Further investigation in this area will evaluate whether this type

of genetic information can help identify patient response to medication, specifically whether interventions aimed at the renin-angiotensin aldosterone system (RAAS), which modulates endothelial dysfunction, such as an ACE/ARB or aldosterone blocker medication.

Future investigation should be aimed at exploring mechanisms of sex differences to provide a platform for optimization of sex-specific therapies if appropriate, through incorporating genomics and pharmacogenomics. Because women and men can have differences in pharmacological respons, sex-specific pharmacogenomics should be included in pharmacological development.

Sex Differences in Thrombosis and Current Thrombolytic Strategy Response: Implications for New Local Delivery Development. ***Alice K. Jacobs MD***

The importance of including women in clinical trials and of reporting sex-specific data cannot be overemphasized. Within the NHLBI PTCA and Dynamic Registries, enrollment of Caucasian men was capped with each wave of the registry and resulted in an enriched sample of women and ethnic minorities. In the 1985-86 registry, in-hospital mortality in women undergoing percutaneous coronary intervention (PCI) was tenfold higher than in men[87]. To further explore this observation, the NHLBI re-opened the registry in 1993-94 to women only, a novel concept that resulted in a study of 583 women[88]. These strategies are models for planning clinical studies to enhance our understanding of potentially important issues specific to women with in CVD.

Given the known differences in the type, treatment, and outcomes of MI in women and men, it is important to explore the basis for sex differences in thrombosis.

Experimental studies reveal a difference in various components of hemostasis between women and men[89]. Several genes coding for proteins involved in hemostasis are regulated by sex hormones, suggesting a molecular mechanism[90] for the observation that estrogens increase the risk of venous thrombosis. The relevance of this finding to arterial thrombosis is not well explored. While aspirin decreases platelet reactivity to a similar extent in women and men, it is not as effective in primary prevention of MI in women in contrast to its superior efficacy in stroke prevention in women in comparison to men (Figure 4). Further investigation regarding mechanisms of sex differences in clinical states of arterial thrombosis are needed.

To place the issue in perspective, it is notable that among the 1.4 million patients discharged with a diagnosis of an ACS in 2005, there were approximately 600,000 with unstable angina, and over 800,000 with a MI[91]. Of the 1.2 million patients discharged with a diagnosis of new or recurrent MI, 60% were men and 40% women, at a cost of $76 billion to the nation[91]. While the incidence of MI is higher in men overall, the risk of death following an MI for women is higher in virtually every study including those in both the pre-and post-reperfusion eras[92, 93]. Vaccarino and colleagues have documented this increase in adverse female mortality is explained by a higher death rate in the younger but not older women in comparison to men (Figure 5)[21]. In patients treated with fibrinolytic therapy in the Thrombolysis in Myocardial Infarction (TIMI)-II trial, significantly higher rates of death and re-infarction were observed in women compared to men at six weeks and one year, even after adjustment for age and co-morbidity[92]. The Enoxaparin and Thrombolysis Reperfusion for Acute Myocardial Infarction Treatment-Thrombolysis in Myocardial Infarction (ExTRACT-TIMI)-25 study demonstrated a

higher incidence of death following reperfusion with fibrinolytic therapy in women (with ST-elevation MI (STEMI)) in comparison to men at all ages[94], and similar findings have been reported in trials of primary PCI for ST-elevation MI (STEMI)[95]. However, the absolute risk reduction in mortality in patients treated with fibrinolytic therapy is similar in women and men. The impact of revascularization on mortality in patients with cardiogenic shock complicating acute MI, is also similar with both women and men benefit from early revascularization[11]. The sex differences in mortality following reperfusion are predominantly explained by baseline differences, including advanced age and greater co-morbidity in women.

In patients treated with unfractionated heparin versus enoxaparin following fibrinolysis in ExTRACT-TIMI-25, the cumulative incidence of death or nonfatal MI at 30 days was significantly lower in both men and women in the enoxaparin group. Of note the absolute risk reduction that was higher in women[94] suggesting that sex differences may dictate a more beneficial response in women for specific therapies. For both women and men undergoing PCI, the clinical benefit of glycoprotein IIb/IIIa platelet receptor blockade with abciximab in terms of 30 day adverse events was similar although women experienced a higher incidence of minor (but not major) bleeding[96]. In patients with ACS not undergoing early coronary angiography, there was a significant reduction in death or MI at 30 days in the group treated with IIb/IIIa receptor blockade compared with placebo. When analyzed separately for women and men, it was noted that men experienced benefit with an odds ratio of 0.81 (95%CI 0.75-0.89), compared to a suggestion of harm among women (odds ratio 1.15, 95% CI 1.01-1.30), while high risk women, defined by an elevated troponin level, derived benefit[69]. Overall women with

ACS and ST elevation MI (STEMI) appear to derive similar benefit from pharmacologic therapy in comparison to men, although they are more likely to have bleeding problems. Prior studies document that women are more likely to be over-treated with anti-thrombotic medication compared to men[97], leading to worse outcomes due to higher bleeding risk. More recently, in ExTRACT-TIMI-25, there was a higher incidence of bleeding with enoxaparin than with unfractionated heparin in both women and men, but a sex difference in bleeding risk was not observed likely because medication doses were adjusted for age and renal function[94].

Women are more likely to present with ACS and less likely to present with STEMI compared to men, yet 30 day mortality or reinfarction with STEMI is higher in women than men[98]. Notably, within non-STEMI patients, there is no sex difference in mortality, and within patients with unstable angina, mortality is lower among women[98]. Although prior studies have observed higher rates of plaque erosion compared with rupture in women[12], they should be interpreted with caution because women with STEMI tend to be excluded from trials of fibrinolytic therapy based on their advanced age. In fact, the TACTICS TIMI-18 investigators evaluated biomarkers in patients with ACS and reported a higher prevalence of markers such as C-reactive protein (CRP) and brain naturetic peptide (BNP), e.g. markers of inflammation and heart failure[24], whereas men had a higher prevalence of troponin and creatine kinase suggesting that myocardial infarction in men was due to plaque rupture.

How do these sex differences impact our therapy? Overall, in patients with ACS, treatment with a routine invasive compared with a selective invasive strategy results in a lower risk of death or MI (odds ratio 0.82 95%CI 0.72-0.93). However, in the studies

that separated results by sex, the benefit of the routine invasive strategy was only seen in men and in high risk women[99, 100]. Moreover, in both women and men, the routine invasive strategy was of benefit in the presence of at least one positive biomarker. However, in women without a positive marker, the invasive strategy was actually harmful[24], whereas among men, there was no difference between the invasive and conservative strategy in the absence of a positive marker.

These data have resulted in the new sex-specific recommendation in the American College of Cardiology/American Heart Association practice guidelines for management of unstable angina and NSTEMI[101]. For women with high-risk features, recommendations for the invasive strategy are similar to those in men, but for women with low risk features, a conservative strategy is recommended. Numerous studies of patients undergoing PCI have reported a higher in-hospital mortality in women in comparison to men and it has been shown that the sex difference in mortality persists in patients with or without STEMI undergoing PCI with stents[102]. It has also been shown that women treated with drug-eluting stents derive similar benefit as men with a decreased incidence of repeat revascularization compared to patients treated with bare metal stents[103].

In summary, the sex differences in thrombosis and outcomes of women with MI and ACS support further investigation of sex-specific therapeutic strategies. Several studies evaluating intra-coronary delivery of IIb/IIIa platelet receptor antagonists with both abciximab and eptifibatide have shown some benefit with respect to decreased bleeding risk in women[104]. Stent delivery of pharmacologic agents, coating of stents, and bioabsorbable stents are potential areas of investigation of relevance to women. Finally,

developing systems of care that increase the number of patients with timely access to primary PCI may be of particular benefit in women, since they have an increased incidence of stroke following treatment with fibrinolytic therapy[105].

Targeting these issues in women provides a unique opportunity for sex-specific innovation. Because women have a greater risk of thrombosis related to estrogen compared to men, as well as more bleeding complications related to anticoagulant treatment, thrombosis management with CVD strategies that include devices should be preferentially tested in women.

Sex Differences in Stem Cell Therapy for CVD. *Dr. Eduardo Marbán*

The area of sex differences in health and disease is ripe for exploration. Scientifically; it is an area that is exploding and may be of particular importance for stem call therapy. A list of sex differences gleaned from the literature is shown in Table 2[106-109]. Additional pre-clinical work conducted by Taylor and colleagues[62, 63] demonstrate important sex differences in endogenous and exogenous PC function that suggest that female PCs promote cardiovascular repair. Prospective human clinical study design can take direction from these pre-clinical studies to maximize regenerative strategy success at this early stage.

Stem cells that are being investigated for use in regenerative medicine are primitive self-renewing multi-potent cells, with multi-potent meaning that the cells have multi-lineage potential. The types of cells that have been considered for regenerative therapy include embryonic stem cells, which have the advantage of being totipotent (e.g., they can actually evolve into all different types of tissues). Nevertheless, there are

concerns such as potential of cells to develop into other tissues, immune rejection and ethical dilemmas involving the destruction of the blastocyst. Therapeutic cloning, that is the production of genetically identical individuals solely for the purpose of generating their stem cells, is probably the only tangible way of using an embryonic stem cell without involving immune reactions. The recent creation of genetically-engineered somatic cells such as fibroblasts, which then acquire embryonic stem cell characteristics (so-called induced pluripotent stem cells, commonly abbreviated as iPS cells), is an exciting new development[110] but comes with all the risks associated with genetically-manipulated cells.

Currently, bone marrow stem cells (allogenieic cells and autologous cells) have been used in over 3,000 heart patients worldwide, and are derived either from the peripheral blood or from resident bone marrow cells. An initial clinical trial using bone marrow mononuclear cells yielded modest improvements in LV ejection fraction in post-MI patients with a good safety profile and an overall decrease in serious adverse events[111]. These results are sufficiently encouraging that they prompt the search for the best delivery method, the best patient population, and the best cell type. In terms of mechanism of benefit, it is questionable whether the cells actually make new heart tissue versus initiating paracrine mobilization of endogenous repair mechanisms.

In the area of organ-specific stem cells, skeletal myoblasts, or more recently, cardiac endogenous cardiac stem cells have been used. Evaluation of endothelial PCs has demonstrated that the number of colony-forming units, as well as a migratory assay in vitro are significantly 50-100% higher in women than in men[106]. Study of MSCs or marrow-derived stromal cells, has suggested that male cells produce more TNF and less

VEGF than female cells[108]. Post-ischemic rat hearts injected with female MSCs had a higher functional recovery than those injected with male MSCs[107]. We studied 80 biopsies from patients of both sexes and found no differences in proliferative capacity, however the study was not designed to elicit such differences[109].

Future work needs to move to exploration of mechanisms to explain these stem cell differences. Females have higher circulating levels of PCs with greater migratory capacity and higher rates of tissue repair. CVD tissue repair strategies should ideally be tested first in females to have the best chance of success for proof-of-concept, followed by testing in the more challenging male models.

Summary

Cardiovascular (CVD) is the leading killer of both women and men. A substantial amount of observational data document that sex differences in CVD exist, and yet mechanistic knowledge, ranging from basic science inquiry to pathophysiological understanding, diagnostics and therapeutics, is limited. The available data implicate a variety of mechanisms to explain why CVD is qualitatively and quantitatively different in women and men; future research should continue to move from observational studies to examination of sex-specific mechanisms in order to develop novel sex-specific therapies, including new devices, tissue engineering, thrombotic strategies, pharmacogenomics and stem cells, to improve outcomes for women and men.

Table 1. Sex Differences in CVD and Implications for Therapies – Summary Points

- **Point 1** - Important sex differences exist in cardiovascular disease (CVD) which impact disease initiation, diagnosis and treatment.
- **Implication** – Research that acknowledges these differences is needed to optimize outcomes in women and men.

- **Point 2** - Atherosclerosis is qualitatively and quantitatively different in women and men; women demonstrate more plaque erosion, more diffuse plaque with less focal artery lumen intrusion.
- **Implication** – CVD strategies that include devices should explore differing anatomical shapes and surfaces as well differing drug coating and eluting strategies.

- **Point 3 -** Bone marrow progenitor cells (PCs) engraft differently based on the sex of the donor cell and the sex of the recipient.
- **Implication** – PC therapeutic studies need to consider the sex of cells of the source and the recipient.

- **Point 4** – Women have a greater risk of thrombosis related to estrogen compared to men, as well as more bleeding complications related to anticoagulant treatment. Several genes coding for proteins involved in hemostasis are regulated by sex hormones.

- **Implications** – Thrombosis management with CVD strategies that include devices should be preferentially tested in women.

- **Point 5** – Women and men can have differences in pharmacological response.
- **Implication** – Sex-specific pharmacogenomics should be included in pharmacological development.

- **Point 6** – CVD progression results from an imbalance of cell injury and repair due to insufficient PC repair, which is impacted by sex differences.
- **Implication** – CVD regenerative strategies should be directed at learning to deliver cells that shift the recipient balance from injury toward repair.

- **Point 7** – Females have higher circulating levels of PCs with greater migratory capacity and higher rates of tissue repair.
- **Implication** – CVD tissue repair strategies should ideally be tested first in females to have the best chance of success for proof-of-concept, followed by testing in the more challenging male models

Table 2. Sex Differences in Stem Cells

- **Endothelial progenitor cells (PCs)**
  - Higher number of colony forming units (CFUs) and greater migratory activity in women than in men.

- **Mesenchymal stem cells (MSCs)**
  - Male cells produce more tissue necrosing factor (TNF), less vascular endothelial growth factor (VEGF) than female MSCs in mice
  - Improved functional recovery in post-ischemic hearts treated with female MSCs relative to male MSCs in rats

- **Cardiac stem cells**
  - No sex differences in proliferative capacity in humans

Appendix

Faculty and Invited Attendees:

Co-Chairs:

Noel Bairey Merz, MD, FACC, FAHA, Director, Cedars-Sinai Medical Center Women's Heart Center

Saralyn Mark, MD, Associate Professor of Medicine and OB-GYN at Yale University and Georgetown University

Faculty:

Barbara D. Boyan, PhD, Professor of Biomedical Engineering at Georgia Tech and Emory and Associate Dean for Research, College of Engineering, Georgia Institute of Technology

Alice Jacobs, MD, Professor of Medicine at Boston University's School of Medicine and Director of the Cardiac Cath Labs and Interventional Cardiology

Eduardo Marbán, MD, PhD, Director of the Heart Institute, Cedars-Sinai Medical Center

P.K. Shah, Director of Cardiology and Atherosclerosis Research, Cedars-Sinai Medical Center

Leslee J. Shaw, PhD, Professor of Medicine at Emory University

Doris Taylor, MD, PhD, Director of the Center for Cardiovascular Repair at the University of Minnesota

Invited Guests:

Christina Anne, Vice President and Global Business Unit Leader of the Women's Health Division at Cook Inc.

Lori Tysdal, Senior Quality Assurance Manager at K2 Corporation, Cook Inc.

Don Rodda, Cook Incorporated, Small Management Group for Engineering at Cook Inc.

Terry McKewen, President of K2 Corporation, Cook Inc.

Sarah Strickler, MD, Medical Director at Men Institute, Cook Inc.

Participants:

Deborah Barbour, MD, practicing cardiologist in Maryland

Dan Berman, MD, Director of Cardiac Imaging, Taper Imaging, Cedars-Sinai Medical Center

JoAnne Eastwood, RN, PhD, Assistant Professor at the UCLA School of Nursing

Morton Kern MD, Associate Chief of Cardiology at University of California Irvine

Joan Kirschner, RN, Research Nurse for the Women's Heart Center, Cedars-Sinai Medical Center

Patrice Nikkens, MD, Medical Officer and Program Director in the Heart Failure and

Margo Minissian, AHP, Cardiology Nurse Practitioner in the Women's Heart Center, Cedars Sinai Medical Center

Elizabeth Murphy, PhD, Heart, Lung and Blood Institute, NIH

Arrythmia Group, National Heart and Lung Institute, NIH

Maura Paul-Labrador, MPH and doctoral candidate, Cardiovascular Epidemiology, Cedars-Sinai Medical Center*

Donna Polk, MD, MPH, Director, Preventive Cardiology, Hartford Hospital

Jonathan Sackier, MD, Professor of Medicine and Surgery at the University of Virginia in Charlottesville

Chrissandra Shufelt, MD, Fellow in Women's Health in the Women's Health Center, Cedars-Sinai Medical Center

Louise Thomson, MBBS, Cardiologist, Taper Imaging, Cedars Sinai Medical Center

Yu-Ching Yang, Biostatistician, Women's Heart Center, Cedars-Sinai Medical Center

*deceased

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Sex Differences in Vascular Disease Prevalence and Risk Factors

Dr. Bairey Merz: With regard to mechanisms, it's clear across a lot of lines of evidence that women of all ethnicity but notably African American females have less obstructive coronary disease yet they have the worst vascular outcomes. What would you say from your data? Are some potential mechanisms that explain this, as we try to go beyond observation of sex differences?

Dr. Shaw: One cannot separate the influence of multiple factors including environmental stressors as well as socioeconomic factors. But, we believe what is most prominent is the degree of co-morbidity. If you look at the minority populations, a greater frequency or clustering of risk factors is notable, with risk factors being more severe and at a decidedly younger age when compared to Caucasian, non-Hispanic cohorts. We have also looked at coronary calcification in patients, and actually revealed that minority patients who have the worst prognosis were those younger subsets with multiple cardiac risk factors.

Dr. Desvigne-Nickens: I just want to share with you some unpublished data from a program in Birmingham, the Tuskegee Legacy study. The program is an educational intervention, and has a population that includes 2/3 of poor, both Caucasian and Black. Notably, 2/3 of the participants who understand hypertension and the importance of

treatment, feel that when the doctor is prescribing medication for them, that they are conducting an experiment on them without their permission. So clearly there's an issue of trust on top of finance, access, and other things.

Dr. Sackier: So you're basically saying that the Black and Hispanic patients are presenting earlier in life.

Dr. Shaw*:* They are presenting earlier in life, correct. On average, minority patient populations are several years younger compared to Caucasian, non-Hispanics, but they do so with a greater risk factor burden.

Dr. Sackier: It's also perhaps worth making the point that the laws in the United States on the need for obligatory post-mortem in the event of sudden death are I think personally, woefully inadequate. And if we did more post-mortems, we'd have a better sense I think of disease prevalence.

Dr. Jacobs: During the past three decades, every study has shown that when women present for coronary angiography or revascularization, they have a higher prevalence of all of the risk factors but a lesser extent of epicardial (perhaps I should now say "focal") , obstructive coronary disease. In the BARI 2D trial, women were treated the same as men with regard to the initial medication and dose, yet they were significantly less likely to reach the target goal for blood pressure, hemoglobin A1C, and LDL cholesterol. So putting those two observations together, it appears that there's something different about the impact of risk factors on the expression of epicardial atherosclerosis in women.

Sex Differences in Atherosclerosis and Response to Intimal Injury

***Dr. Bairey Merz*:** It may be that men are not diagnosed with Tako-Tsubo syndrome perhaps because they do not survive due to gender differences in ventricular fibrillatory thresholds.

***Dr. Shah*:** That's an excellent point, and it appears that loss of estrogen in older women actually may create a setup for Tako-Tsubo syndrome because estrogen regulates adrenergic receptors in a negative inotropic direction, protecting the basal myocardium from the stress-related injury. Accordingly, this protective mechanism would be absent in men, suggesting the hypothesis that when men suffer a similar stress-induced event, they don't have protected basal constrictors to keep them alive.

***Dr. Jacobs*:** We seem to be seeing more and more Takasubo's syndrome. Do you think the prevalence has increased or we are just more aware of the syndrome (given the invasive approach to acute myocardial infarction)?

Dr. Shah: If you look at the literature in the 1960's, there are many reports of acute myocardial infarction in women with angiographically normal coronary arteries. I think the syndrome has been there for a long time. We just are beginning to recognize it better because more patients are having acute angiography, and the title of Tako-Tsubo syndrome captivates everybody, so we're likely recognizing it more often. I don't think the prevalence has really changed, just recognizing it as a unique entity more often is more likely.

***Dr. Murphy*:** My question focuses on the hormone progesterone. We always focus on estrogen and the relationship between men and women with that hormone.

Dr. Shah: We have looked at matrix metalloproteinase (MMP) expression in macrophages in response to oxidized LDL exposure and observed that estrogens actually

downregulate oxidized LDL induced MMP expression, while progesterone actually reversed the effect of estrogens on that potentially protective mechanism (unpublished data).

Dr. Mark: In regard to carotid arteries, there have been some reports that women more frequently have restenosis post-endarterectomy. Can you take into account what might be some of the mechanisms for that? Regarding vascular injury, dissection, and angioplasty, what are sex differences with regard to vascular repair and restenosis?

Dr. Shah: First of all, restenosis of carotid endarterectomy is relatively uncommon in general. It is not nearly as common as post angioplasty restenosis in the coronaries. And that suggests that the biology of the response is likely to be different. Why it is more in women, I don't really have a clear-cut explanation, although because the size of the arteries is relatively smaller, any regrowth is likely to be more obstructive in women compared to men.

Dr. Kern: The restenosis rates in men and in women at least in the small vessels, have fairly similar outcomes, but these are mostly in the elderly women where much of the estrogen-related sex difference likely falls away.

Dr. Taylor: There are data emerging that mesenchymal stem cells play a role, by differentiating into smooth muscle cells and contribute to restenosis. The role of transforming growth factor beta (TGF-beta) combined with the sex differences between men and women, can push more of those cells down a smooth muscle pathway. A picture that is emerging involves endogenous vascular repair mechanisms that differ in men and in women.

Sex Difference in Vascular Cellular and Tissue Engineering

Dr. Mark: Can an adipocyte differentiate into a myocyte or osteocyte, and what are the sex differences and age implications?

Dr. Boyan: Liposuction adipocytes are unfortunately not an adequate source of progenitor cells and stem cells, however subcutaneous fat works well. We have isolated MSCs from subcutaneous fat and find it to be a good source of stem cells with potential to become chondrocytes. It is a less effective source for osteoprogenitor cells in our hands. We are currently working to determine whether the loss of this osteogenic progenitor population has got to do with age and sex of the donor and if this cell population can be enriched through simple procedures that could be used in a clinical setting.

Dr. Taylor: Are you saying then that those are not mesenchymal progenitor cells but they're already committed to a given lineage?

Dr. Boyan: No, they are MSCs but not all MSCs have the same differentiation potential; it depends on the tissue type and sex of the source. We have successfully induced MSCs in subcutaneous fat to become neurons, osteoblasts, chrondrocytes, and adipocytes, but the fact that they can go down a pathway does not say that every MSC does so equally. What we've been doing is actually quantifying the relative proportion of cells with a given phenotype using markers that are a little different from the classic panel of markers, to try to determine how many cells in a population of MSCs really have the potential to go down any particular pathway. The fat source we're using, subcutaneous fat, definitely has osteogenic potential, but it's not as effective as bone marrow-derived cells.

Dr. Bairey Merz: Women have more osteoporosis than men and I think your data support a mechanism to explain that sex difference. With regard to angiogenesis does your data show that women are conversely better at angiogenesis?

Dr. Boyan: We haven't compared the males and the females that way yet. I don't have an answer to that. That would be really interesting to get at that piece of data.

Dr. Bairey Merz: What additional proportion of your research enterprise would be needed to add routinely sex comparative studies?

Dr. Boyan: I think it would take a minimum of another 50%.

Progenitor Cell and Cytokine Regulation of Vascular Tissue: Implications for Repair/Restenosis and Local Drug Delivery

Dr. Bairey Merz: It seems pretty clear from these data that there is sex differences in both mice and humans that favor better progenitor cells and better vascular repair in women compared to men, until older ages. Does it make sense to hypothesize that women may be inherently better capable of processes such as tissue growth, repair, and new cell formation because of their reproductive charge biologically to produce a fetus?

Dr. Taylor: I would absolutely say that I think that's true, and some of the most interesting data that I've seen in preclinical studies describes the changes that occur in cardiovascular load and hypertrophy with pregnancy. We know that there is a need for increased angiogenesis, cell number, and all of the things that we associate with positive repair during pregnancy. Having markers to evaluate that did not exist five years ago will allow us to dissect out what cells are involved, what cytokines are involved, and see if

similar algorithms that are present in disease failure and pregnancy need for positive repair.

Dr. Murphy: Do cytokines change with oophorectomy in females?

Dr. Taylor: Oophorectomy makes female mice worse with regard to change in circulating cell populations. We also have found, similar to Dr. Boyan's work, that the bone marrow progenitor cells express estrogen. Furthermore, we found that depending on where animals were in the estrous cycle, the number of cells that you got could differ.

Dr. Murphy: Estrogen level in female, even when low, is still higher than it is in a male, suggesting that a relatively high estrogen level may be needed for protection.

Dr. Taylor: This may have implications for the cell therapy trials that are underway using autologous cells versus allogeneic cells, and actually introduces the question of whether or not female cells would be better delivered into males.

Dr. Bairey Merz: Given everything that you know right now, should here be a preference for trying to test cell therapy preferentially in women?

Dr. Taylor: I think that's a critical question. I direct the bio-repository for the Cardiovascular Cell Therapy Research Network (CCTRN) in the U.S., and evaluation of the inclusion criteria of the various cell therapy studies' suggests that we will treat a minority of women. I am concerned that we will see more negative responses in the men, and if we were to pick a system that is more favored to respond, we would pick women to study first, then the harder case, and men going forward. How do we address this issue in the large multi-network studies going forward?

Dr. Bairey Merz: We need to start by addressing issues of policy and funding. That's usually how anything happens.

Sex Differences in Medication Response and Pharmacogenetics

Dr. Boyan: We did a study with platelets and I'm kicking myself, because all of the volunteers were young male medical students. Platelets are just loaded with the growth factors that initiate the wound healing response, and not just the acute inflammatory response but also the migration of the MSCs to the site. Has anyone looked at the male or female composition of this?

Dr. Bairey Merz: Not that I know of. There is a lot of basic work that needs to be done.

Dr. Mark: Could you discuss perhaps the pharmacogenetic component for what we saw in HERS trial, with the women on hormone therapy?

Dr. Bairey Merz: There are ancillary publications from the HERS that suggests differing HDL responses to the hormone therapy according to genotype, and that if you add up all the risk factors associated with the hormone therapy including the elevations in triglycerides, that it really explains the HERS outcomes, specifically that it is a mixed bag of risk factor impact in an older population with established CAD[77].

Dr. Shaw: Can I ask about treating ischemia? I think sometimes in those women, since you don't have any obstructive coronary artery disease; women are often not treated with anti-ischemics. Would it be reasonable to try to retest them to see if ischemia is resolved?

Dr. Bairey Merz: We are doing two randomized control trials right now, including a ranolazine placebo-controlled crossover study where we're using extent and severity of ischemia using our cardiac magnetic resonance imaging. That's ongoing, and then our second study is a multicenter randomized controlled NHLBI-sponsored trial (Pepine PI) of eplenerone with the outcome of repeat coronary reactivity testings.

Dr. Mark: How do you feel about the coronary calcium testing?

Dr. Shaw: First of all, the coronary calcium scores are not adjusted for arterial size. And so any given amount of coronary calcification would encumber a larger arterial area on a women compared to a man which would then be associated with a greater risk. Our data do demonstrate a sex difference whereby women are at relatively greater risk for a given coronary calcium score.

Dr. Boyan: As women aged, their kidney function also decreases, and they actually produce lower levels of 1,25-dihydroxy vitamin D3 than males do. And then there's this whole issue of the entire population being low with their levels of this vitamin D metabolites. Do you see that as impacting this calcium score at all, specifically that women may be under-calcified?

Dr. Shaw: In the younger women, they have relatively lower calcification, and in older women, they have relatively more calcification. More research is needed in this area.

Sex Differences in Thrombosis and Current Thrombolytic Strategy Response: Implications for New Local Delivery Development

Dr. Bairey Merz: The data are compelling that low risk women don't benefit from an invasive strategy in the acute coronary syndrome, however the data also do not support primary angioplasty for low-risk men. Why the gender difference in the guidelines.

Dr. Jacobs: The sex-specific recommendation concerning the routine invasive strategy in women with acute coronary syndromes was based on the signal that there might be some harm for low risk women

Dr. Mark: Intra-coronary stent delivered therapy, would you believe that we would have the same benefit if it were another arterial system, such as the periphery? What about the venous system?

Dr. Jacobs: There are few data evaluating sex-specific outcomes of stents in the periphery arterial or venous. However, the technologies are exploding and there are different agents that potentially could be delivered on stents that might be specific to the healing process in women versus men. For example, due to the high bleeding risk in women, development of a stent designed to deliver local antithrombotic therapy may be particularly useful.

Dr. Kern: Peripheral vascular disease presentations are not similar to the acute coronary syndromes, that is, peripheral vascular plaques do not rupture and thrombose suddenly, such that we do not have acute sudden limb syndromes as they do with coronary syndromes.

Dr. Kern: Peripheral vascular disease presentations are not similar to the acute coronary syndromes, that is, peripheral vascular plaques do not rupture and thrombose suddenly, such that we do not have acute sudden limb syndromes as they do with coronary syndromes.

Dr. Jacobs: Another consideration is that the collateral circulation is more developed in periphery, and the prevalence of collateral circulation is higher in women than men, although that is uncorrected for size and diabetes status.

Dr. Kern: A drug-eluting stent may be applicable to women and men at differing stages of life related to age and hormonal status. So part of our thinking needs to be break that

down. I think that part of the research that could be applied to these types of problems should also be stratified by phase of life.

Sex Differences in Stem Cell Therapy for Vascular Disease

Dr.Bairey Merz: What are the plans in this Phase 1 / 2 clinical trial recruitment, e.g. given what might be some sex differences in these cells, should you preferentially recruit women?

Dr. Marbán: Well, it's a good question. We certainly are not going to exclude either men or women in this phase 1 study. On the basis of the data that we have right now with our own stem cell population, the data are insufficient for us to prespecify sex as an independent variable, but we are developing potency assays that may allow us to glean relevant differences. We will be mindful of tracking whether there's any predictive value of these in vitro assays and whether there's any indication that there might be sex-based differences. This study design is autologous, and therefore involves female cells into females and male cells into males, and not a crossover study.

Dr. Bairey Merz: Dr. Shah, you are the principal investigator here of a study of biological therapy that is conducted exclusively in women with refractory angina.

Dr. Shah: Yes, there are data from two Phase 2 trials suggesting that adenovirus-mediated FGF delivery has a greater clinical benefit in women than in men, and that formed the rationale for the Phase 3 trial of which we are a clinical site. The trial is limited to women only.

Dr. Boyan: Consider randomizing enough subjects in your trial to evaluate women and men separately.

Dr. Marbán: The Phase 1 study is planned for 24 patients including a placebo arm and two doses, so we won't be able to probe sex differences, but we anticipate a Phase 2 study to follow with about 200 patients and there would be a very relevant issue to keep in mind.

Concluding Comments/Panel Discussion. ***All Faculty, Guests and Participants***

Dr. Mark: We've heard a lot about sex differences and now want to address a translational perspective to improve the health of men and women from a vascular perspective.

Dr. Tolstrup: Are the vulnerable plaques' composition different in men and women with regard to the biomarkers discussed today such that we could identify them with a new imaging type modality?

Dr. Shaw: We can't yet quantify atherosclerosis by its subcomponents of non-calcified or mixed plaque to the same extent that we can measure calcified plaque.

Dr. Taylor: We have started working with antibodies that you can conjugate to microbubbles visualizable by ultrasound and are looking at the ability to get those microbubbles to track to specific cell types based on cell surface markers, and then follow those to the lesion. We see the changes in the plaque differ in males and females and that the composition of the macrophages and the CD45(+) cells differ in males and females.

Dr. Boyan. There is a growing amount of nanotechnology involved with imaging. As I understand it, the endothelial coating is not consistent over the plaque. This could be

picked up relatively easily using some of these nanotechnology imaging agents without necessarily being toxic to cells if they were targeted properly.

Dr. Bairey Merz: Should device companies be thinking about vehicles for injection delivery, sustained delivery slow-release devices? What do you think?

Dr. Taylor: The CD 34-coated stent that in theory was going to recruit progenitor cells to its surface and get re-endothelialized more quickly than a bare metal stent or a drug-eluting stent did not do a good job of recruiting those cells, suggesting this is a complex area. Conversely, data suggest that local estrogen decreases plaque erosion, I could imagine a bi-drug delivery type phenomenon with something like estrogen on one side and an endothelial cell recruiting agent on the other side for example.

Mr. Rodda: There are technologies with devices where you can put a drug in, specifically we can place the drug on the site and remove a device, or put something on a stent and leave it both the drug and stent there. The intermediate is to put a drug on and have it there for some timeframe and then have the delivery mechanism removed or absorbed by the body.

Ms. Strickland: I know that estrogen was one of those things that were used coated stents and I don't know why it didn't pan out. It may have been related to how well it came onto the stent.

Dr. Bairey Merz: It could also be that the estrogen trials were negative in men, and the device was tested primarily in men.

Dr. Murphy: The SERMs, or selective estrogen modulators that may be used to selectively target more than estrogen.

Dr. Jacobs: There are a number of catheter-based technologies to identify the vulnerable plaque, but none that I know of looking at sex differences. If there is a difference in re-endothelialization following injury, then we should be looking at sex differences in stent thrombosis in patients treated with drug-eluting stents.

Dr. Taylor: What we need to do is stop smooth muscle proliferation but not endothelial proliferation, so looking at more targeted drugs would be better.

Dr. Boyan: Maybe we don't need the fancy drug alluding stents if we modified the material surface. Some of the modifications are simple, and they really just have to do with hydrophilicity and have nothing at all to do with sticking a drug on. Consider exploring how to get the cells to migrate on and set up shop? I would also be more specific about which metal. They could be other kinds of metal treatments.

Mr. Rodda: One thing that's interesting is reading the explant reports which would discuss what cellular growth occurred, where there would be thrombus and the like. There were differences on the different materials, yet no clear understanding of why the different materials had different histology.

Dr. Boyan: Although we should point out that the explants were devices that for whatever reason didn't work well.

Mr. Rodda: But maybe didn't have anything to do with the explant, these patients were very ill prior the procedure. The explants often occurred years after the original placement.

Dr. Barbour: One thing I think we really have to keep in mind in the coronary circulation where you have so much smaller an area to work with, we obviously need a treatment that will promote endothelialization without promoting thrombogenicity.

Dr. Boyan: At the nano structural level, 100 nanos and it's rough already. If you gave the endothelial cells a shape instead of necessarily thinking about sticking another bioactive molecule on top, that you could get to a product much faster and just as efficiently by giving them a physical environment that they can identify with. You could do this by keeping the metal hydrophilic throughout the processing method, not allowing it to passivate at all.

Dr. Barbour: Dose surface charge something that can be affected at the vascular wall without a permanent device? Can there be something used on the surface of a balloon that is blown up against a wall affect surface charge or affect surface properties such that you would promote endothelial growth, and then you deflate the balloon and remove it?

Dr. Boyan: That's not a bad idea to do something like painting, putting the balloon, letting it deposit a kind of paint on the surface and then withdrawing the balloon.

Dr. Bairey Merz: If investigators had to submit grants that were 50% higher because they had to meet criteria of doing sex difference analyses, what would the impact be of that?

Dr. Taylor: Unless grant reviewers understand it, we're all going to be hosed if we're the only ones that do it.

Dr. Bairey Merz: It has to be mandated.

Dr. Nikkens: That fact is that on the books now it is required that studies not only include adequate number of subpopulations but the analysis plan include these subgroups, well powered, and that cost should not be an issue not to do this. But of course often there is no subgroup analysis planned, not adequate numbers, and if the real costs were put in there, the NIH wouldn't accept it because the cost itself becomes prohibitive. These

deliberations however might underscore that the best science, however, or most efficient science to study is sex specific. Studying female only, at least in the case for repair, is a resounding theme from the data reviewed.

Dr. Mark: What about the FDA and the development of a device with regard to sex differences?

Ms. Strickland: In our large study to approve an abdominal aortic aneurysm device, we were concerned that there were not be enough women in that study, and so developed with the FDA a female registry within that study, by studying thoracic aortic aneurysms.

Dr. Boyan: There has been a sufficient assessment of women in most of the studies; however sponsors are not required to power studies sufficiently to make a labeling claim. The studies of devices tend to be 200 to 400 patients, but in a few cases it has been sufficient to where the companies have gone back and argued for a labeling claim to use a device in only one or the other sex. I was invited to explain to the FDA that men are different from women, and it was a really eye-opening experience.

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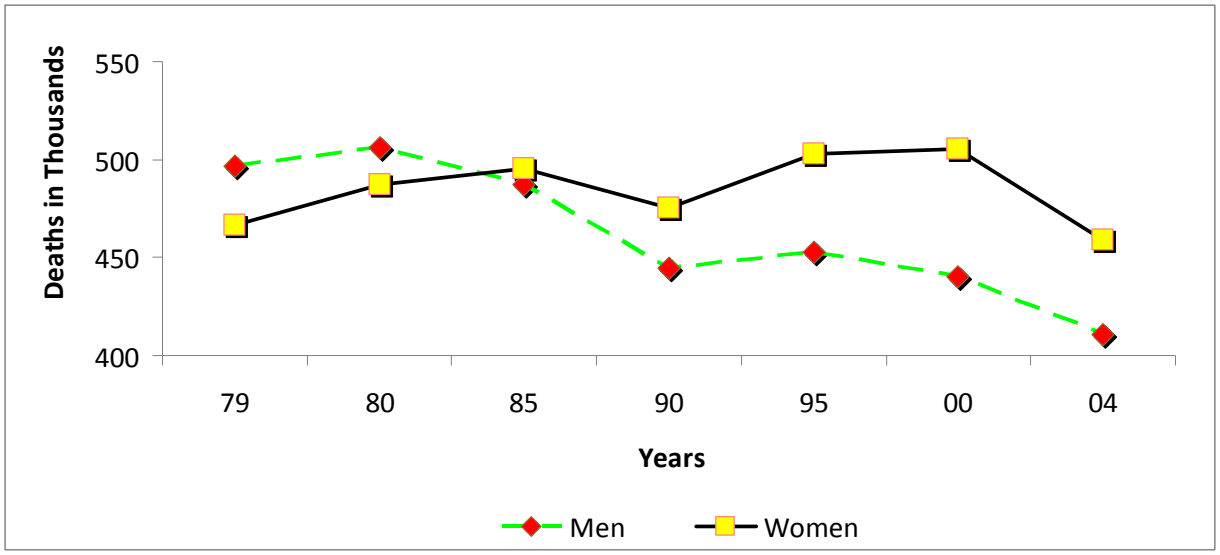
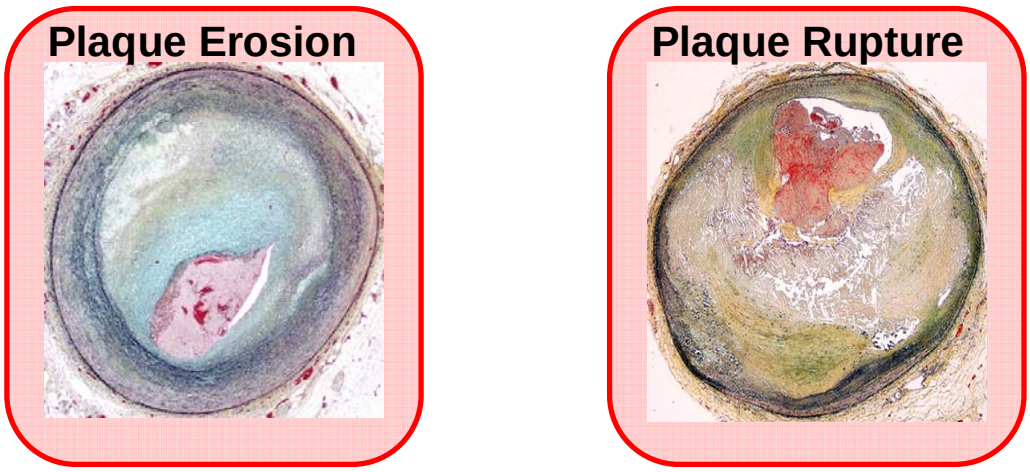
Figure 1. CVD mortality trends in women and men



CVD disease mortality trends (US: 1979-2004).
Source: NCHS and NHLBI (2008).

Figure 2. Sex differences in atherosclerotic plaque disruption



Younger women: Plaque erosion:

- Thrombus over a base rich in smooth muscle with proteglycan-rich matrix (necrotic core - often absent): 40% of thrombi in sudden cardiac death

Men and older women: Plaque rupture:

- Thin fibrous cap over large necrotic core Infiltrated by foamy macrophages: 60% of thrombi in sudden cardiac death

Source: Burke Circulation 1998;97:2110-2116.

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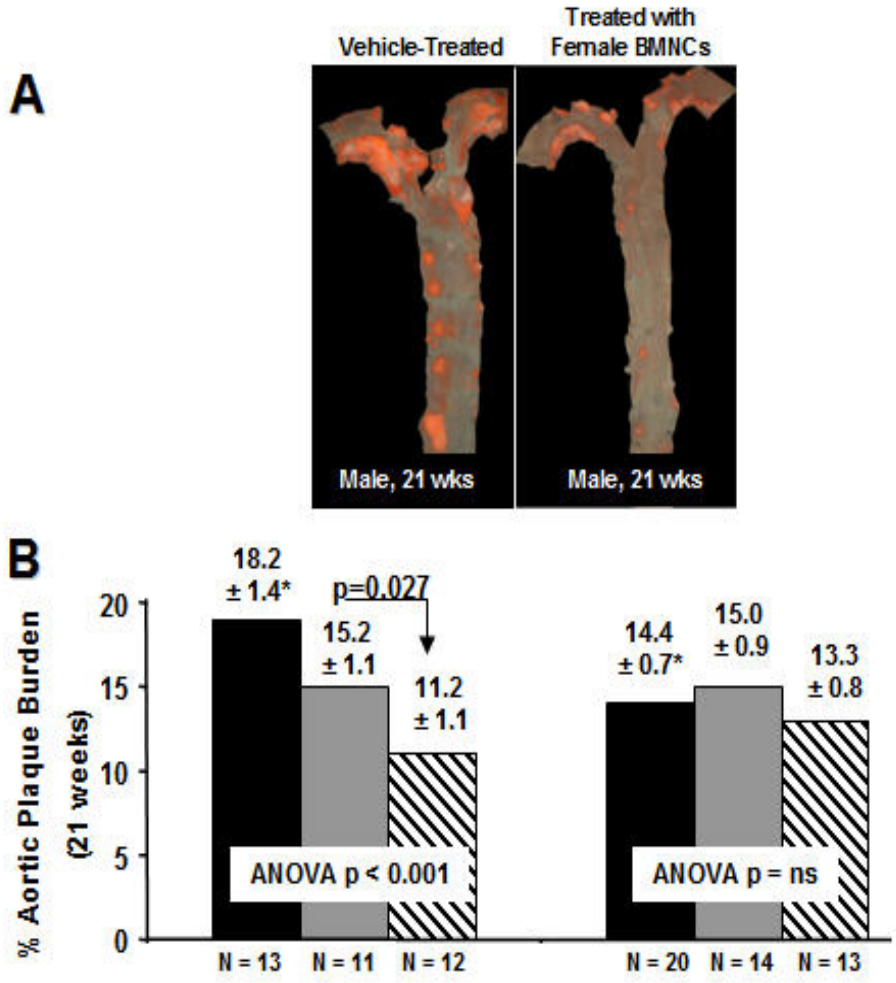
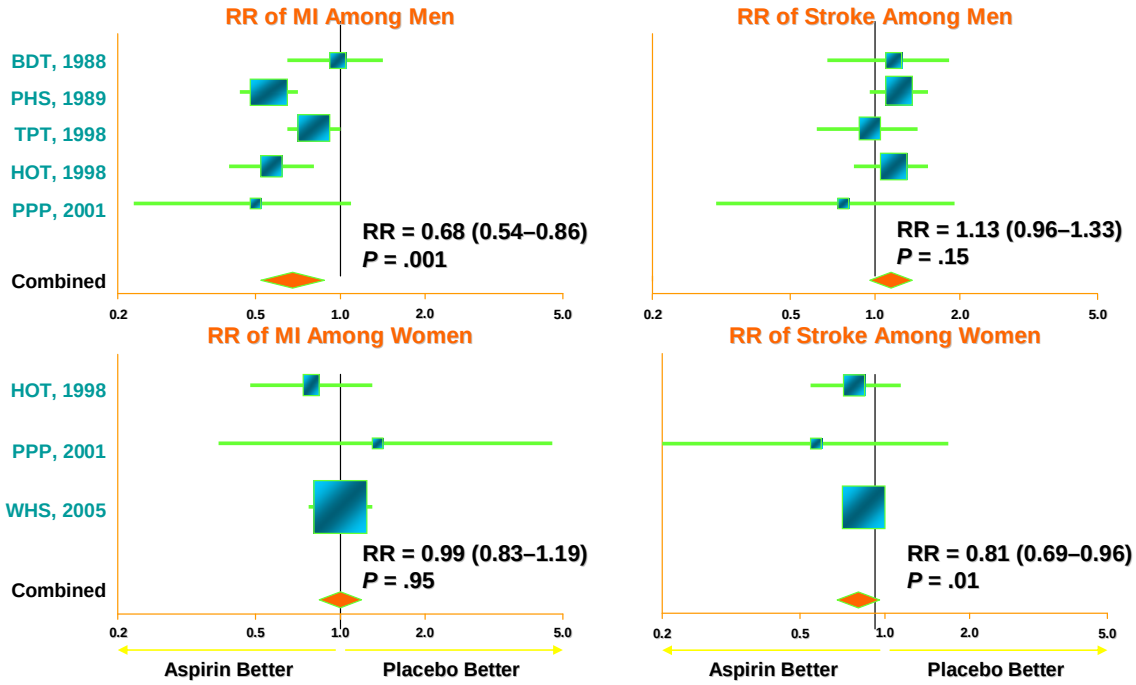
Figure 3. Female PCs are more beneficial for reducing atherosclerotic plaque burden



Sex-dependent attenuation of plaque growth after treatment with bone marrow mononuclear cells

Nelson et al, Circ Res 2007 (with permission)

Figure 4. Sex differences in aspirin therapy for CVD prevention



Ridker, P. et al., *N Engl J Med* 2005; 352:1293-204.

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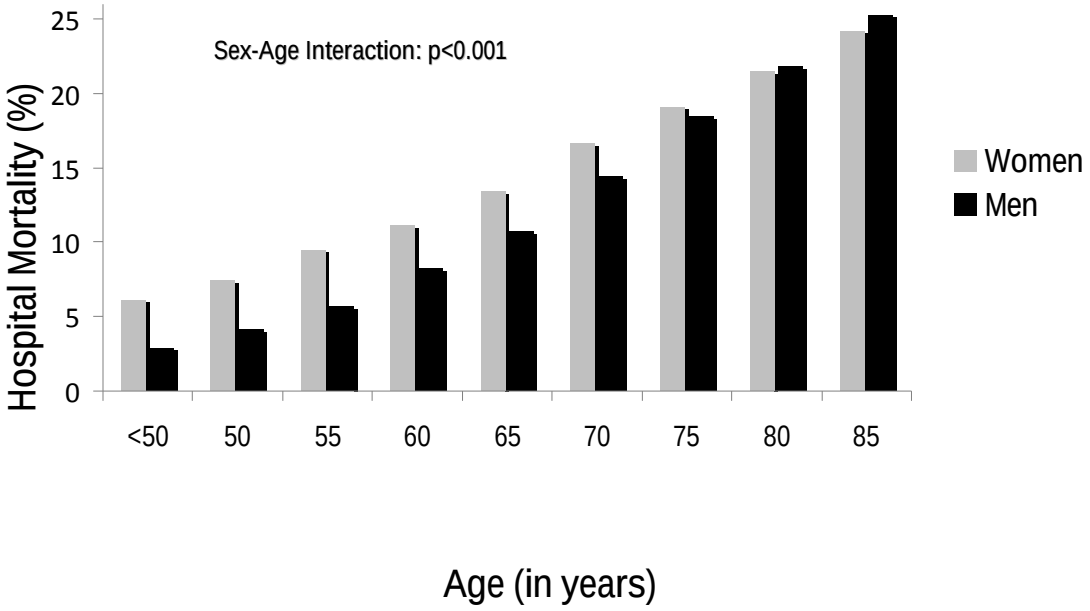
Figure 5. Sex differences in acute MI outcomes



Source: Vaccarino N Engl J Med 1999;341:217

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