Sex differences in vascular endothelial function and health in humans: Impacts of exercise

Daniel J Green[1,2], Nicola D Hopkins[2], Helen Jones[2], Dick HJ Thijssen[2,3], Thijs M.H. Eijsvogels[2,3], Bu B Yeap[4]

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[1] School of Sport Science, Exercise and Health, The University of Western Australia
[2]Research Institute for Sport and Exercise Sciences, Liverpool John Moores University
[3]Radboud Institute for Health Sciences, Department of Physiology, Radboud University Medical Center, The Netherlands
[4]School of Medicine and Pharmacology, University of Western Australia and Department of Endocrinology and Diabetes, Fiona Stanley Hospital, Western Australia,

Name and address for correspondence

Daniel J Green
Winthrop Professor
School of Sport Science, Exercise and Health
The University of Western Australia
Nedlands, Western Australia

Phone: +61 (0) 8 6466 2361
Email: danny.green@uwa.edu.au

NEW FINDINGS

What is the topic of this review?

This brief review discusses potential sex differences in arterial function across the age span with a special emphasis on oestrogen and testosterone effects on the vascular endothelium.

What advances does it highlight?

We discuss the relationship between the impacts of sex hormones on arterial function and health in the context of epidemiological evidence pertaining to the menopause and ageing effects. Studies performed in humans are emphasised, alongside insights from mechanistic animal studies. Findings suggest that the combination of exercise and hormone administration should be potentially synergistic or additive in humans.

ABSTRACT

This brief review presents historical evidence for the purported impacts of male and female sex hormones on the vasculature in humans, including effects on macro- and micro-vascular function and health. Impacts of aging on hormonal changes and artery function are considered in the context of the menopause and puberty. Physiological data are presented alongside clinical outcomes from large trials, in an attempt to rationalise disparate findings along the bench-to-bedside continuum. Finally, the theoretical likelihood that exercise and hormone treatment may induce synergistic and/or additive vascular adaptations is developed in the context of recent laboratory studies that have compared male and female responses to training. Differences between men and women in terms of the impact of age and cardiorespiratory fitness on endothelial function are addressed. Ultimately, this review highlights the paucity of high quality and compelling evidence regarding the fundamental impact, in humans, of sex differences on arterial function and the moderating impacts of exercise on arterial function, adaptation and health at different ages in either sex.

INTRODUCTION

Sex may be the most impactful of any of the individual differences between humans and it is certainly the most universally relevant. For example, recent findings relating to systematic distinctions between men and women in drug metabolism have led the FDA to release warnings pertaining to differential dosing regimens and sex specific approvals (FDA, 2014). The historical bias towards studies of men in clinical, and indeed physiological, research has also generated guidance and recommendations regarding sex balance and sub-analysis in future clinical trials (FDA, 2014).

Sex differences can be narrowly defined as differences due to the sex chromosome or sex hormones. This brief review focusses on sex differences in endothelial function and, in particular, studies of the impact of sex hormones on the vasculature. It emphasizes studies performed in humans and, where pertinent, informs these with reductive and mechanistic experiments. There are implications for understanding distinctions in human physiological function, including adaptations to the stress of exercise.

WHAT GOT US INTERESTED IN SEX DIFFERENCES?

Data from the Framingham Heart Study published 40 years ago largely reinforced the "universal clinical impression" that clinical manifestation of atherosclerotic cardiovascular diseases is extremely rare in younger women, compared to men of similar age (Kannel *et al.*, 1976). The incidence of cardiovascular disease (CVD) by age and sex suggested that pre-menopausal women had fewer cardiovascular and coronary events compared to men, and also compared to post-menopausal women of the same age (Figure 1). Since these differences occurred largely independently of traditional risk factors, changes in estrogen at the menopause were implicated. Later analysis further suggested that women who underwent bilateral oophorectomy were at elevated cardiovascular risk compared to those who had natural menopause and that administration of estrogen decreased risk in both groups (Colditz *et al.*, 1987). Studies such as these prompted physiological experiments aimed at identifying the impact of menopause on artery function *in vivo*.

IMPACT OF THE MENOPAUSE ON ARTERY FUNCTION: PHYSIOLOGICAL EXPERIMENTS

Celermajer and colleagues (Celermajer *et al.*, 1994) published data from 103 men and 135 women with no risk factors for CVD which indicated that conduit artery flow-mediated dilation (FMD), an endothelium-dependent and largely nitric oxide (NO) mediated functional response to increased luminal shear stress (Green *et al.*, 2014) that predicts CV events (Ras *et al.*; Inaba *et al.*, 2010; Green *et al.*, 2011), declined in men at an earlier age than women and that a steep decline in the latter coincided with time of menopause (Figure 2). No decline in vascular smooth muscle function (glyceryl trintrate GTN responses) was evident in either group with age, suggesting that the functional impairment was isolated to endothelial cells. These data indicating progressive reduction in FMD, but not in GTN, were supported by cross-sectional work that compared FMD between pre- and post-menopausal women (Jensen-Urstad & Johansson, 2001) or across stages of the menopause (Moreau *et al.*, 2012a). The endothelial impairment was significantly correlated with reduced levels of estradiol and independent of other CVD risk factors (Moreau *et al.*, 2012a). Recent work from Moreau *et al.* found that administration of tetrahydrobiopterin (BH4; a cofactor for endothelial NO synthesis) improved FMD and carotid artery compliance in post-menopausal women, suggesting that BH4 may contribute to the vascular changes observed after menopause (Moreau *et al.*, 2012b).

Intriguingly, other non-invasive measurements of vascular function, including arterial stiffness, demonstrate a linear relationship with ageing in both sexes (Engelen et al., 2013). A study that characterised the mechanical properties of the human aorta across different age groups in men and women, found that the age-related increases in stiffness (collagen and elastin) of the aorta were smaller in women than in men (Åstrand et al., 2011), a finding ascribed to the impact of sex hormones. Taken together, these data suggest that estrogen status may have specific impacts on arterial endothelial function, rather than more structural vascular indices.

CHANGES IN ARTERY FUNCTION ACROSS THE MENSTRUAL CYCLE: PHYSIOLOGICAL EXPERIMENTS

If female sex hormone fluctuation impacts endothelial function then, logically, differences in arterial responses may be apparent across the menstrual cycle. In an early study, Hashimoto *et al.* assessed FMD and GTN responses in men and pre-menopausal women at three distinct phases of their cycle

(Hashimoto *et al.*, 1995). When serum estradiol was low, FMD was comparable to that in male subjects, whereas responses were enhanced in the follicular phase when estradiol was higher. GTN responses were unaffected, suggesting a specific effect of estradiol on the endothelium. Whilst this study was performed using rudimentary equipment and the FMD changes are remarkably large, the general finding of fluctuations across the menstrual cycle has been replicated by several laboratories. One comprehensive study, by Williams *et al.*, reported findings with several independent measures of vascular physiology (Williams *et al.*, 2001). FMD, acetylcholine (ACh) administration to skin microvessels via iontophoresis, and whole body arterial compliance were all consistently impaired during the early luteal phase. Pulse wave velocity was the exception to this pattern and it has also been reported that arterial compliance, assessed using augmentation index, is lower during the luteal phase of the menstrual cycle (Robb *et al.*, 2009). These findings are somewhat in keeping with the data above regarding structural arterial indices. The menstrual cycle may also be associated with fluctuations in the plasma levels of the vasoconstrictor ET-1, with higher levels observed during the menstrual phase (Polderman *et al.*, 2000). In the whole, available evidence suggests that vascular endothelial function fluctuates with hormonal changes that occur across the menstrual cycle, although there is less certainty regarding measures of arterial stiffness.

IMPACTS OF ESTROGEN ON ARTERY FUNCTION: MECHANISMS

Recent detailed reviews have described the molecular mechanisms associated with the impacts of estrogen (i.e. 17β-estradiol or E2) on vascular function and the endothelium (Knowlton & Lee, 2012; Chakrabarti *et al.*, 2014). These include the generation of NO and prostacyclin, promotion of endothelial repair and regeneration, anti-inflammatory and anti-oxidant effects. Impacts are mediated via genomic (short-term) and non-genomic (longer-term) pathways and depend upon receptor sub-type. ERα- and ERβ-receptors are expressed in human endothelial cells, both within endothelial cells and on their membrane (especially ERα), with beneficial anti-atherogenic effects largely mediated via activation of the ERα sub-type. There is some evidence for expression of these receptors in men, as well as women, possibly reflecting a role for estrogen generation from a testosterone pathway. Physiologically, a major role for estrogen is maintenance of vasodilation mediated via NO generation through eNOS-phosphorylation as well as adaptive genomic upregulation

of eNOS protein. A recent study by Tarhouni and colleagues (Tarhouni *et al.*, 2013) demonstrated an essential role for E2 and ERα in flow-mediated remodelling of resistance arteries, suggesting potential synergistic impacts of endothelial shear stress and estrogen signalling in terms of vascular adaptation following exercise and training *in vivo* (Figure 3). Animal studies indicate that both ageing *per se*, and the menopause, impact on estrogen signalling. Ageing impacts via pro-inflammatory and oxidative stress effects on signalling, whilst progressive estrogen withdrawal impacts receptors and post-receptor pathways. These findings inform the observation that peri-menopausal women express better outcomes from estrogen supplementation than older post-menopausal women (see *Timing Hypothesis*, below), the latter being exposed to pro-atherogenic inflammatory cascades (Knowlton & Lee, 2012). Finally, it has been reported that oral and transdermal administration of estrogen in recently menopausal women increase circulating nitrates, but they have no impact upon endothelin-1 or prostacyclin metabolites (Ylikorkala *et al.*, 1998; Maffei *et al.*, 2006).

Endothelin-1 (ET-1) is a powerful endothelium-derived vasoconstrictor implicated in the age-related reduction in resting vascular tone (Thijssen *et al.*, 2007). Studies, largely performed in animals, have demonstrated lower plasma levels of ET-1 and/or increased expression of ET-1 mRNA levels in females (Lekontseva *et al.*, 2010; Ojeda *et al.*, 2014). This sex difference may relate to estrogen, since ovariectomy increases ET-1 mRNA expression in the female rat and this effect can be reversed by estrogen supplementation (David *et al.*, 2001). Also, in humans, supplementation with estradiol in post-menopausal women lowers ET-1 plasma levels (Lekontseva *et al.*, 2010).

ESTROGEN SUPPLEMENTATION: BENCH VERSUS BED

The Framingham data, Nurses' Health Study (Grodstein *et al.*, 1996) and the vascular physiology literature summarised above provided a basis for optimism regarding potentially beneficial impacts of estrogen supplementation on endothelial function and, consequently, cardiovascular health in humans. Indeed, these studies provided some rationale for the widespread clinical adoption of hormone replacement therapies (HRT) in the late 20th century. In 2002, the initial results of the Women's Health Initiative (WHI) (Writing Group for the Women's Health Initiative, 2002), a randomised controlled trial (RCT) of 16,608 women (50-79 yrs) with an intact uterus comparing

conjugated equine estrogen (CEE) plus progestin to placebo, reported that per 10,000 women, the group receiving hormones exhibited 7 excess coronary heart disease (CHD) events, 8 more strokes, 8 more pulmonary emboli and 8 more breast cancers. Whilst there were 6 fewer colorectal cancers, 5 fewer hip fractures and no impacts on all-cause mortality, the study concluded that the regimen of CEE and P should not be initiated or continued for primary prevention of CHD. Subsequent analysis of the affiliated WHI RCT involving administration of CEE alone in women with prior hysterectomy revealed 12 more strokes per 10,000 women versus the placebo group, but 6 fewer hip fractures, 7 fewer breast cancers and no significant difference in CHD (The Women's Health Initiative Steering, 2004). Collectively, these data, along with results of the HERS trial (Grady *et al.*, 2002), had a profound clinical impact on the use of hormone therapies in the preventive context for women without menopausal symptoms.

Some rationalisation of the disparate findings from physiological experiments indicating beneficial impacts of estrogen on vascular function and adaptation, with these RCTs illustrating no CV benefit, was possible as a result of primate studies (Clarkson *et al.*, 2013). Administration of estrogen soon after ovariectomy substantially decreased atherosclerotic plaque formation, whereas delayed administration (equivalent to 6 years in humans) resulted in no plaque inhibition (Figure 4). In a complimentary study it was found that monkeys with pre-existing atherosclerosis received no benefit from CEE administration, whereas progression was almost eliminated in those with low levels of atheroma (Clarkson *et al.*, 2013). On the basis of these and other findings, Clarkson proposed the *"Timing Hypothesis"* which suggests that the beneficial impacts of estrogens in terms of atherosclerosis and cardiovascular disease are considerable during pre- and peri-menopausal years when estrogen receptors are intact and there is limited prevailing atherosclerosis (*usually* younger women), whereas administration of estrogen to those with *a priori* evidence of atherosclerosis (*usually* older and post-menopausal women) confers no beneficial effect and, may even be detrimental (Clarkson, 2007). This proposal gained credence from molecular studies cellular biology studies (Mendelhson & Karas, 2005) along with re-analysis of the WHI which suggested some benefit of CEE in terms if CAD and stroke in younger but not older women (Manson *et al.*, 2013).

Recently, trials that were designed to directly address the timing hypothesis have been reported. The Kronos Early Estrogen Prevention Study (KEEPS) was designed to assess the effects of two types of hormone therapy (oral and transdermal) compared to placebo in healthy, recently menopausal women at low risk for cardiovascular disease. Progression of carotid wall thickness, a surrogate measure of structural atherosclerotic vascular adaptation, was unaffected by either treatment and coronary calcium was also unchanged (Hodis *et al.*, 2014). Digital tonometry (EndoPat), a relatively new surrogate for vascular function, was also not different between groups. Importantly, it was concluded that *"Variability in RHI* [Endopat derived outcome measure] *was unexpectedly high and serves as a caution in the utility of digital tonometry to detect subtle changes in risk factor profile or treatment effects as would be needed to add value to existing cardiovascular risk algorithms"* (Kling *et al.*, 2015). It is therefore difficult to interpret the vascular findings of the KEEPS trial and its implications regarding the timing hypothesis. Another study, the ELITE trial, recently reported preliminary findings. This trial studied 643 women with prior hysterectomy, 271 of who were <6yrs post-menopausal (~55 yrs), with the remainder >10yrs (~65 yrs). The rate of progression in carotid wall thickness was significantly reduced, versus placebo, in the early administration group but not in those administered estrogen late after the menopause. This preliminary report stated that the hormone treatment effect on progression significantly differed between the early versus late post-menopausal groups (P<0.007) (Hodis *et al.*, 2014), adding support to the timing hypothesis in terms of vascular measures. It is, however, appropriate to await the full peer-reviewed publication of the results of this trial. The current clinical equipoise (2015) is that hormone therapy, especially with CEE, remains a reasonable option for management of menopausal symptoms in women, for a short period following onset of the menopause. Further clinical trials of the timing hypothesis will be necessary before the use of estrogen can be recommended for prevention and chronic disease management.

TESTOSTERONE AND ARTERIAL FUNCTION IN HUMANS

Whilst there is robust evidence that very high doses of testosterone (T) and anabolic steroids have detrimental impacts on cardiovascular risk factors, CV events and endothelial function, the impact of lower doses that "normalise" circulating T levels is less clear.

Several studies have reported improvements in vascular function, including endothelial function, as a result of acute administration of T *in vivo*, particularly in those subjects with low initial circulating concentrations of T. Collins, Ong and colleagues measured coronary artery diameter and flow using quantitative angiography and Doppler flow wires in men aged ~60 yrs with low normal circulating T and established CAD (Webb *et al.*, 1999). They reported dose-dependent increases in coronary artery diameter and flow in response to T infusion. A lack of synergistic effect with acetylcholine co-infusion led the authors to conclude that the mechanism was unlikely to be receptor-mediated. It was speculated, on the basis of previous *in vitro* experiments, that the pathway of the vasodilator effects of T might involve activation of ATP-sensitive K^+ channels on vascular smooth muscle (VSM) cells, although recent evidence implicates multiple mechanisms, including calcium channels (Kelly & Jones, 2013). Collins and Ong *et al.* subsequently published data indicating that acute T administration enhanced brachial artery FMD in a similar group of male subject and proposed that a flow-mediated endothelium-dependent mechanism was responsible (Ong *et al.*, 2002), a conclusion relevant to potential combined or synergistic impacts of exercise and testosterone administration in humans (see exercise section below). In heart failure patients, acute administration of T increased cardiac output and decreased peripheral vascular resistance, suggesting a beneficial impact on afterload alongside impacts on functional capacity (Figure 5) (Pugh *et al.*, 2003). Taken together, these studies indicate that T, at physiological doses, has potentially beneficial impacts on vascular function in males.

Although several studies suggest beneficial effects of longer term treatment with T on outcomes such as muscle strength and functional capacity, very few have measured vascular adaptation. Kang *et al.* reported that oral administration of T for 12 weeks significantly enhanced brachial FMD, relative to placebo administration in men with CAD (Kang *et al.*, 2002). Since vasodilator responses to a NO agonist also improved, the improvement may be related to smooth muscle cell function. Although some studies have reported no improvement in vascular function following treatment (Sader *et al.*, 2003), it generally appears that T is capable of inducing vasodilator effects through endothelium-dependent and –independent pathways, with the former both genomic and non-genomic in nature (Kelly & Jones, 2013). Some impacts of T may also be mediated via its aromatisation to estrogen, through pathways described above (Yeap, 2015).

VASCULAR IMPACTS OF TESTOSTERONE: CLINICAL TRANSLATION

Whilst it has been reported that T declines in males after the age of 30 at a rate of approximately 1-2% per year, the question as to whether this decline is age or lifestyle related is currently unclear (Araujo *et al.*, 2004). Nonetheless, the popularity of T has markedly increased in recent decades, reflecting the notion that it may be a restorative male hormone which reverses age- and obesity-related decline. The impact of T on cardiovascular endpoints and its safety remain controversial topics.

The Testosterone in Older Men with Mobility Limitations (TOM) study, performed in 209 frail older men with chronic diseases, reported 23 CV events in the T treatment group, compared to 5 in the placebo arm (Basaria *et al.*, 2010), whilst a subsequent retrospective study of 8,709 Veterans Affairs patients with low T reported increased risk of adverse outcomes in the treatment group (Vigen *et al.*, 2013). The latter study was criticised, on the basis of controversial analysis methodology (event rates), flaws in the randomisation process (some women were included) and group bias caused by administration of T after infarction in some subjects (Morgentaler & Lunenfeld, 2014). Nonetheless, these studies recently prompted the FDA to publish a caution about using T products for low T due to ageing, requiring labelling of such products to provide information regarding a possible increased risk of heart attack and stroke (03-03-2015).

Contrary to the evidence above, several cohorts have indicated that low T in men is associated with increased coronary events and stroke (Yeap, 2015). In addition, meta-analyses have generally reported no excess of adverse effects from T therapy (Yeap, 2015). Finally, a recent report found no evidence for differences in coronary calcium or carotid wall thickening following 3 years of T administration, compared to placebo, in older men with low baseline T (Basaria *et al.*, 2015). In contrast with the FDA statement above, the European Medicines Agency (representing EU states), concluded in November 2014 that there exists "*no consistent evidence of an increased risk of heart problems with testosterone medicines"*. A thorough review of the conflicting studies in the field of T

therapy and administration was recently published (Yeap, 2015), making it clear that large RCTs with CV endpoints will ultimately be required to settle the question of the clinical efficacy of T in men.

In conclusion, it appears that the purported vascular benefits of T therapy are dependent upon the dosage used, the route of administration (gels may be better than frequent injections) (Layton *et al.*, 2015), the age of the subjects, their atheromatous substrate and the metabolism of testosterone *in vivo,* including aromatisation. The recently published data of Barua *et al.* of 83,010 men with low testosterone levels, normalization of testosterone levels using T was associated with lower mortality, fewer MIs, and strokes. The significant benefit was only observed in those patients in whom the dose is sufficient to normalize the T levels (Sharma *et al.*, 2015).

IS IT JUST ABOUT THE HORMONES?

Enthusiasm regarding the potential beneficial effects of E2 on artery function and health led to the administration of estrogens to men. Collins *et al.* (Collins *et al.*, 1995) administered ACh into the coronary arteries of post-menopausal women (59 yrs) and men (52 yrs) with coronary disease, with and without E2 co-infusion. In the women, ACh caused coronary vasoconstriction that was reversed by administration of E2, whereas in men E2 had no impact upon ACh responses. This lack of beneficial impact in men mirrors findings from the Coronary Drug Project, in which administration of estrogen to men was associated with some evidence of excess cardiovascular events (Coronary Drug Project Research, 1974). In contrast to these findings, Sader and colleagues reported improvement in FMD responses in healthy young men with T implants who were also administered estradiol (Sader *et al.*, 2001). These improvements in artery function occurred in the absence of effects on GTN administration, indicating that the impact was endothelium-mediated. This group (McCrohon *et al.*, 1997) and others (New *et al.*, 1997) have also reported that male to female transsexuals administered prolonged high dose estrogen exhibit enhanced FMD responses compared to control male subjects. The latter studies are complicated to interpret due to the impact of confounding variables, but they suggest that longer term estrogen administration may impact on endothelial cell estrogen receptors.

Studies of post-menopausal women receiving estrogen, who were also administered T, suggested that both FMD and vascular smooth muscle function are enhanced (Worboys *et al.*, 2001). However, it is clear that young women with polycystic ovary syndrome (PCOS), characterised by high circulating T concentrations and advanced atherosclerotic development, possess impaired endothelial function, arterial stiffness and responsiveness of endothelin receptors (Paradisi *et al.*, 2001; Carmina *et al.*, 2006; Armeni *et al.*, 2013; Wenner *et al.*, 2013; Sprung *et al.*, 2014) and it has been reported that genetic female-to-male transsexuals taking high dose androgens exhibit impaired vascular smooth muscle reactivity (McCredie *et al.*, 1998).

These studies suggest that, whilst higher doses of estrogens in men and T in women detrimentally impact vascular function and health, lower doses may have some beneficial impacts. The balance between benefit and risk is therefore related to dose (Sharma *et al.*, 2015), subject age, the presence of other CV risk factors or pre-existing atherosclerosis, and the route and duration of administration. There are also complex *in vivo* relationships between T and E2, and their metabolites (Yeap, 2015), which may modulate the ultimate vascular impact of sex hormones *in vivo*.

DOES EXERCISE TRAINING IMPACT ON SEX DIFFERENCES?

The findings presented above indicate that both E2 and T have impacts of vascular function, through endothelial and smooth muscle cells mechanistic pathways. It is therefore tempting to speculate that the combination of exercise training, which enhances endothelial and vascular function and modifies arterial structure (Green *et al.*, 2004), and sex hormone administration, may induce additive or synergistic effects. A recent study by Tarhouni (Figure 4) indicating that structural remodelling induced by repetitive increases in arterial blood flow and shear stress in rats is estradiol- and ERα-dependent, supports this notion (Tarhouni *et al.*, 2013). Studies indicating that T administration enhances smooth muscle cell function (as well as endothelial function), whilst exercise induces shear stress mediated improvement in endothelial function (Tinken *et al.*, 2010), theoretically holds the promising of targeting both cell lines in responsible for the control of vascular tone and remodelling.

There are few studies in humans that have specifically addressed the question of sex differences in the impact of exercise training and those that are available must be considered proof-of-principle studies. Black *et al.* studied a small number of older men (~58 yrs) and women (60 yrs) before and after 24 weeks of training and observed improvements in the FMD and compound (FMD/GTN) vascular function responses of the women, but not the men (Black *et al.*, 2009). Changes in popliteal and brachial artery wall thickness, lumen diameters and W:L ratios that were sex-specific were also observed in this cohort (Green *et al.*, 2010). Somewhat in contrast, Pierce *et al.* observed improvements in FMD in older men, but not postmenopausal women, after 8 weeks of training (Pierce *et al.*, 2011). The small numbers in both studies, differential time course of training (Tinken *et al.*, 2008) and population factors may go some way to explaining the disparity. Moreau *et al.* recently randomly assigned 48 postmenopausal women who were not on HRT to one of three groups: oral E2, transdermal E2 or placebo. Following 12 weeks, all participants undertook a further 12 weeks of treatment during which endurance exercise was added. Brachial artery FMD increased as a result of E2 treatment (both groups), but not in the group who received placebo. The addition of training made no difference in the placebo group, but was additive in the E2 groups (Moreau *et al.*, 2013). This synergistic effect of HRT+ exercise suggests that E2 is required for the beneficial effects of exercise on the endothelial function in postmenopausal women. Finally, Sprung and Jones have recently reported that exercise training has consistent and beneficial impacts of both conduit (Sprung *et al.*, 2013a) and microvascular (Sprung *et al.*, 2013b) function in women with PCOS and high T concentrations.

Evidence pertaining to other vascular measures is less promising. A meta-analysis of exercise effects on PWV combining 42 randomised controlled trials of ≥4 weeks (1627 participants) revealed no significant impact of sex across these studies (Ashor *et al.*, 2014). However, this type of analysis has obvious limitations compared to studies directly comparing the impact of exercise training between both sexes.

SUMMARY

Theoretical evidence and the findings of studies that have assessed the impacts of E2 and T on vascular function suggest that the combination of exercise and hormone administration should be

potentially synergistic or additive (Figure 6). To date, no studies have been specifically designed to examine questions such as whether distinct phases of the menopause or periods of T decline with age in men predispose to greater or less impactful effects of coincident exercise training. The rate of T prescriptions in ageing men has increased dramatically over the last decade, reflecting less than rigorous prescribing habits grafted onto the unproven concept of a restorative hormone that preserves health. Increasing support for the Timing Hypothesis suggests a possible return to estrogen supplementation soon after the menopause for some women. Renewed interest in personalized approaches to medicine, including exercise as medicine, should foster further studies of sex differences and the impact of sex hormones on exercise responses in both sexes. At present, these fundamental questions of individual variation between sexes remain largely unanswered.

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Author Contributions

All authors contributed to the conception or design of this review, the consideration of studies included, the drafting of the manuscript and its critical revision. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Finally, all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Figure Legends

Figure 1. Incidence of cardiovascular disease by age and sex in the Framingham study (above). In the lower panel, CVD incidence is presented in pre- and post-menopausal women of similar age, and men (Kannel *et al.*, 1976).

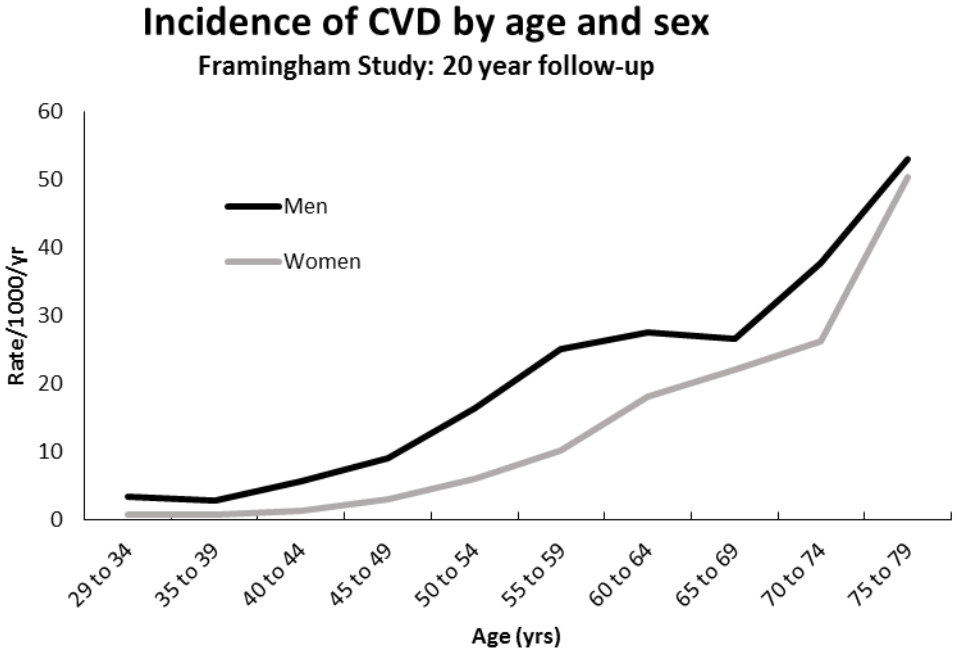
Figure 2. Flow mediated dilation (FMD) a measure of endothelium- and nitric oxide (NO)-mediated conduit artery vasodilation in humans. Decreased function is apparent earlier in men than in women. Lack of impact of age on glyceryl trinitrate (GTN) responses suggest a primary endothelium-mediated impairment with age. Data based on (Celermajer *et al.*, 1994).

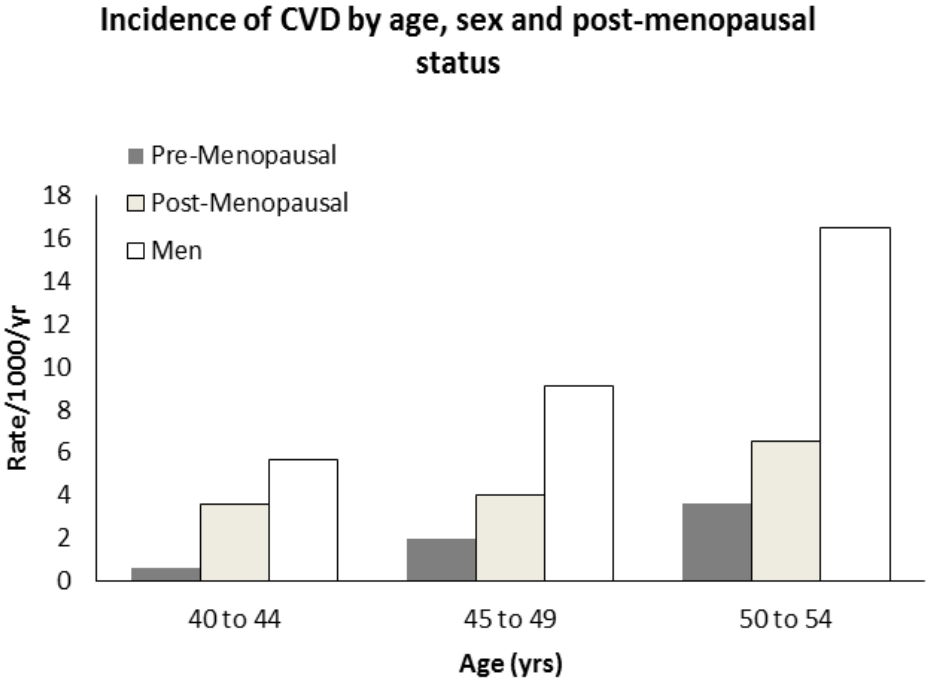
Figure 3. Data from rats indicating that high flow induces arterial remodelling, that is not apparent in ovariectomized animals, unless they received supplemental estrogen (left). Further experiments revealed that high flow failed to induced remodelling in mice lacking the ERα receptor. These experiments indicate that chronic increases in flow induce structural remodelling of arteries that is endothelium- and NO-mediated and also dependent upon estradiol and ERα receptor stimulation. Data derived from (Tarhouni *et al.*, 2013)

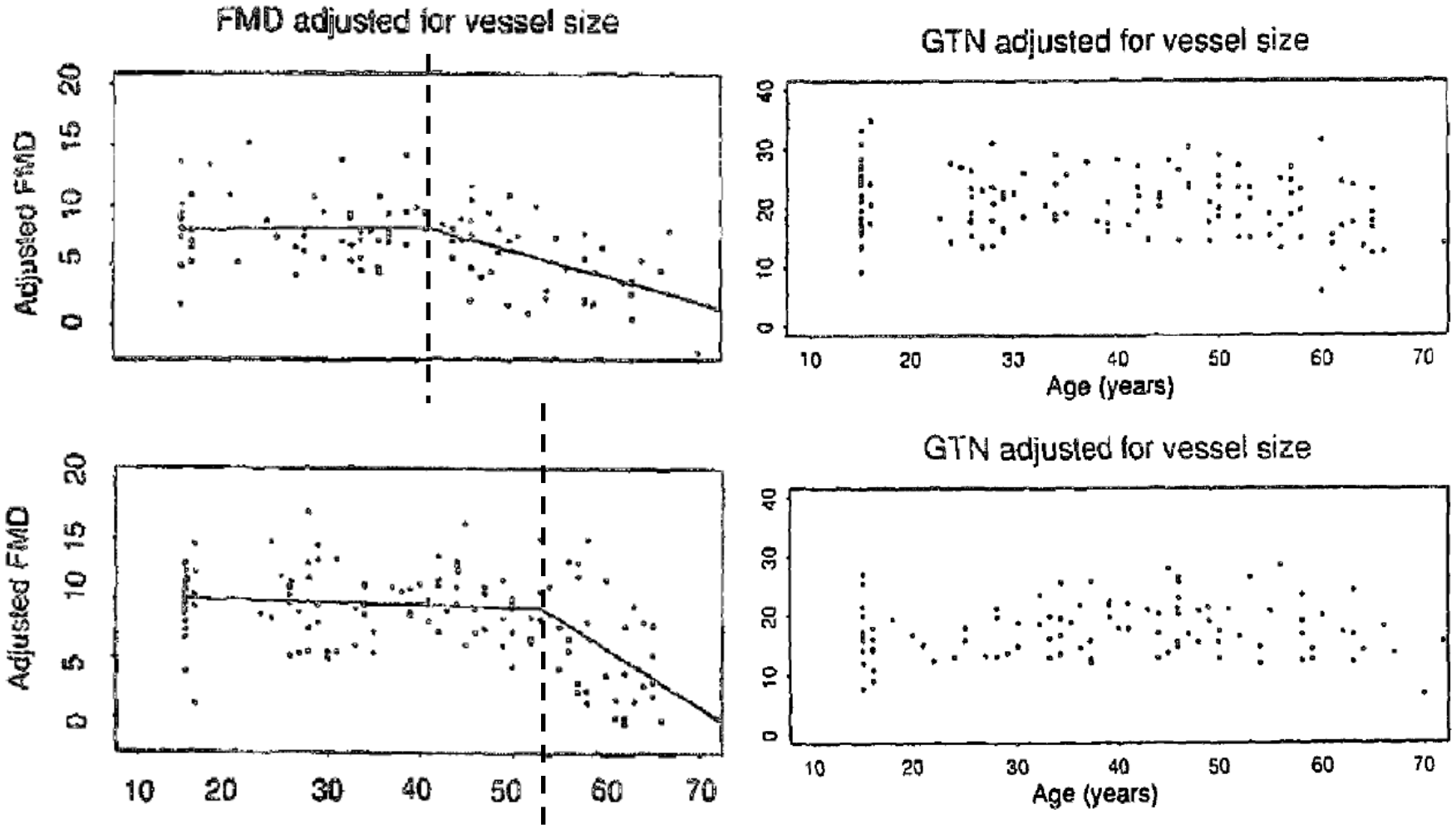
Figure 4. Primate data underpinning the "timing hypothesis". Coronary plaque size is diminished in animals treated with conjugated equine estrogen (CEE) soon after surgically-induced menopause, whereas no effect is apparent if treatment is delayed (upper panels). This effect is likely to be related to the degree of underlying atheromatous substrate, rather than age *per se*, since estrogen treatment of monkeys with low plaque burden is effective, whereas those with high plaque burden show no benefit. These and other data generated the "timing hypothesis", which holds that that the beneficial impacts of estrogens are apparent during pre- and peri-menopausal years when receptors are intact and there is limited pre-existing atherosclerosis (younger women), whereas administration of estrogen to those with *a priori* evidence of atherosclerosis (older post-menopausal women) confers little benefit. Data are derived from (Clarkson, 2007).

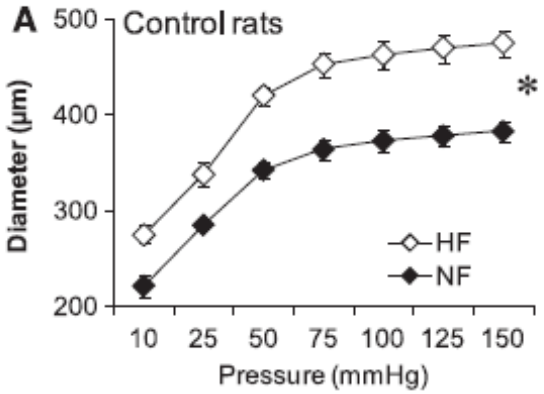
Figure 5. The acute impact of testosterone administration in patients with chronic heart failure. Increases in the bioavailability of T (upper panel) are associated with increases in cardiac index in the presence of diminished total peripheral resistance. Testosterone administration at an appropriate dose may exert beneficial systemic effects on haemodynamics by modulating vascular function. Data are derived from (Pugh *et al.*, 2003).

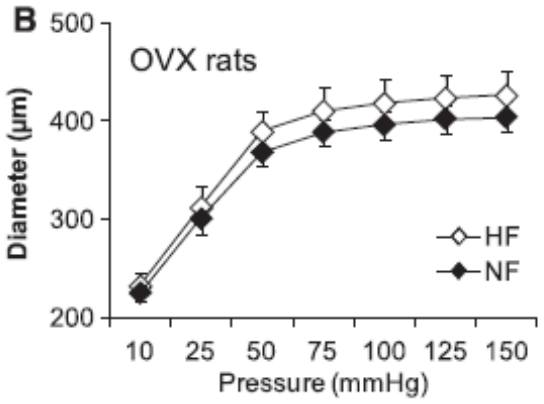
Figure 6. Summary of proposed interactions between sex hormones and the impacts of exercise training on vascular and endothelial function in humans.

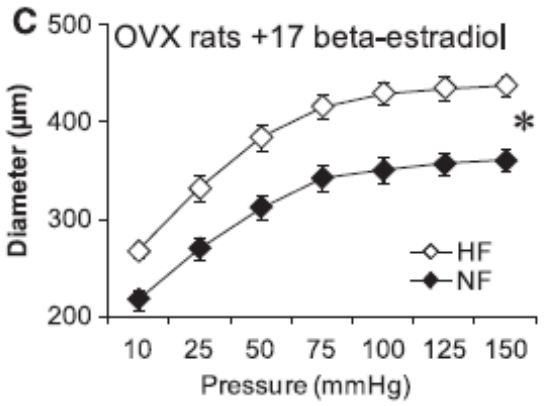

Incidence of CVD by age and sex
Framingham Study: 20 year follow-up
Rate/1000/yr
60
50
40
30
20
10
0
Men
Women
29 to 34
35 to 39
40 to 44
45 to 49
50 to 54
55 to 59
60 to 64
65 to 69
70 to 74
75 to 79
Age (yrs)

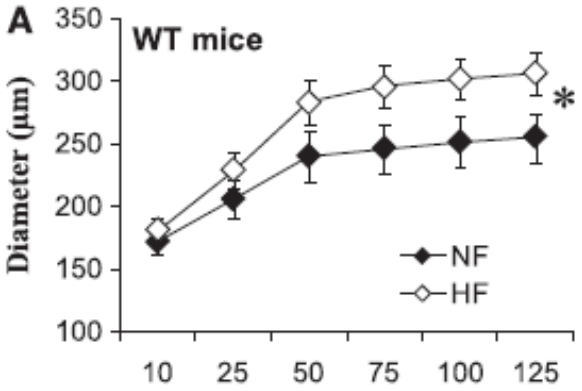

Incidence of CVD by age, sex and post-menopausal status
Pre-Menopausal
Post-Menopausal
Men
Rate/1000/yr
18
16
14
12
10
8
6
4
2
0
40 to 44
45 to 49
50 to 54
Age (yrs)

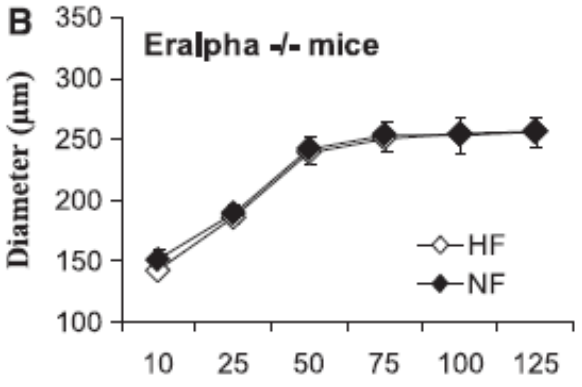

FMD adjusted for vessel size
Adjusted FMD
0
5
10
15
20
GTN adjusted for vessel size
0
10
20
30
40
10
20
30
40
50
60
70
Age (years)
Adjusted FMD
0
5
10
15
20
10
20
30
40
50
60
70
GTN adjusted for vessel size
0
10
20
30
40
10
20
30
40
50
60
70
Age (years)

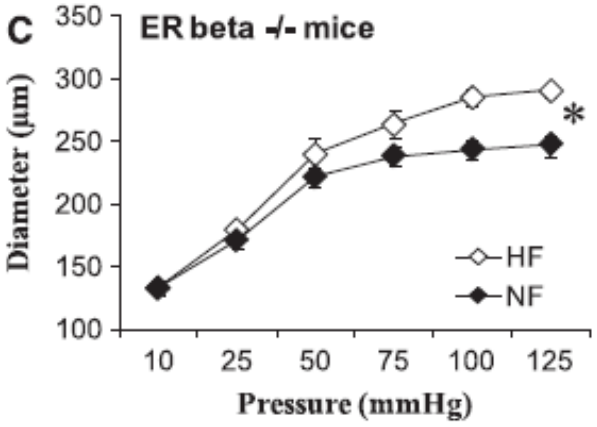

A
Control rats
Diameter (µm)
500
400
300
200
HF
NF
*
10 25 50 75 100 125 150
Pressure (mmHg)

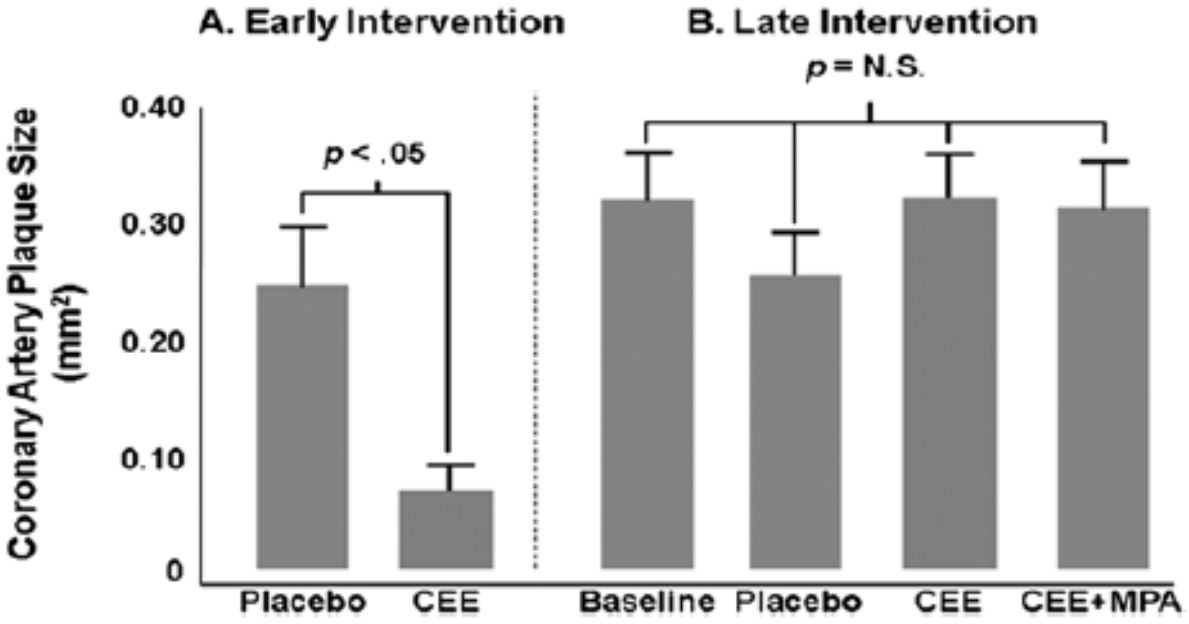

B
OVX rats
Diameter (µm)
500
400
300
200
HF
NF
10 25 50 75 100 125 150
Pressure (mmHg)

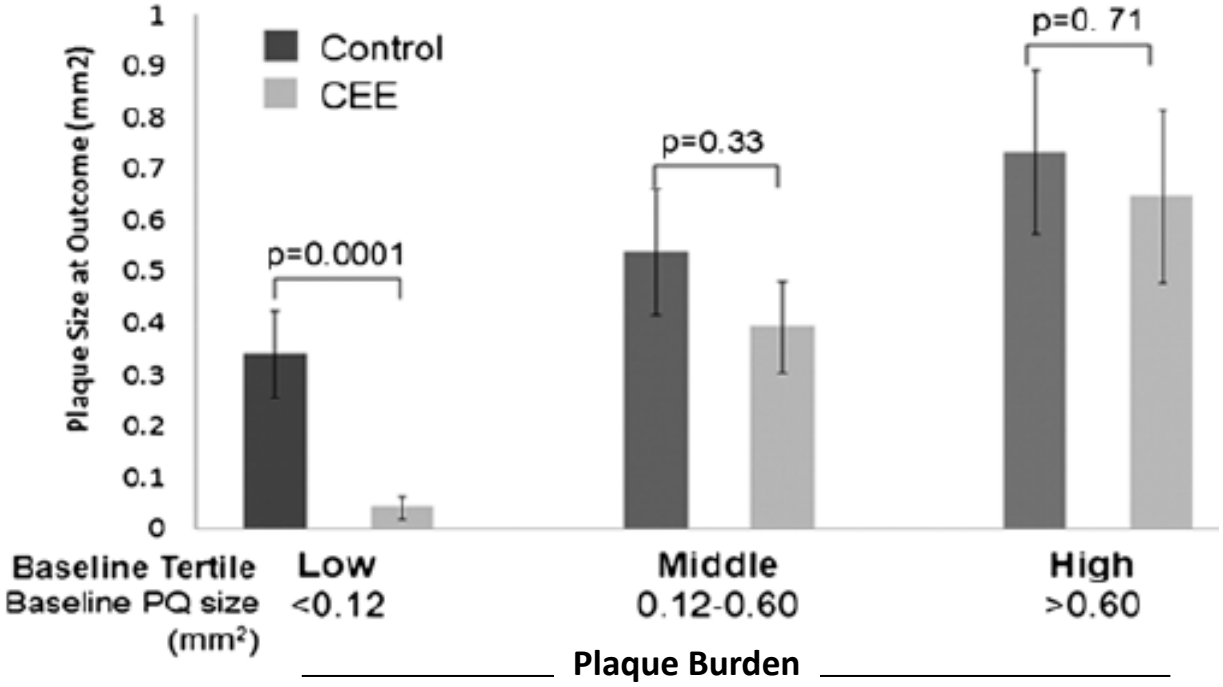

C
OVX rats +17 beta-estradiol
Diameter (µm)
500
400
300
200
HF
NF
*
10 25 50 75 100 125 150
Pressure (mmHg)

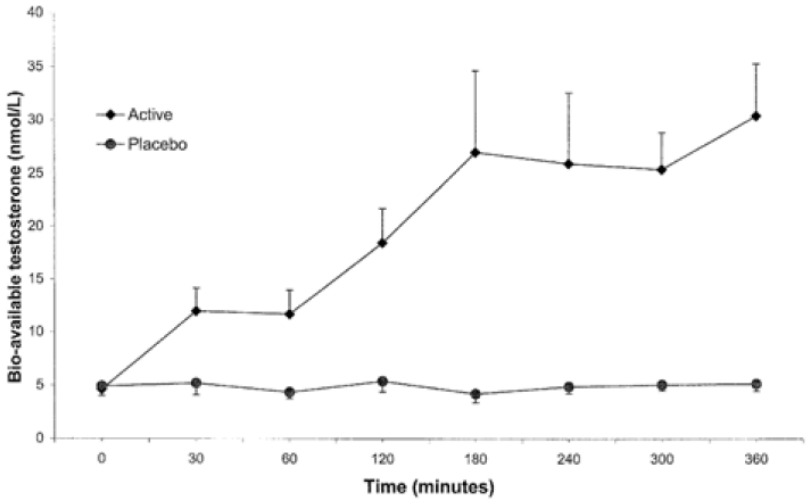

A
WT mice
Diameter (µm)
350
300
250
200
150
100
NF
HF
*
10 25 50 75 100 125

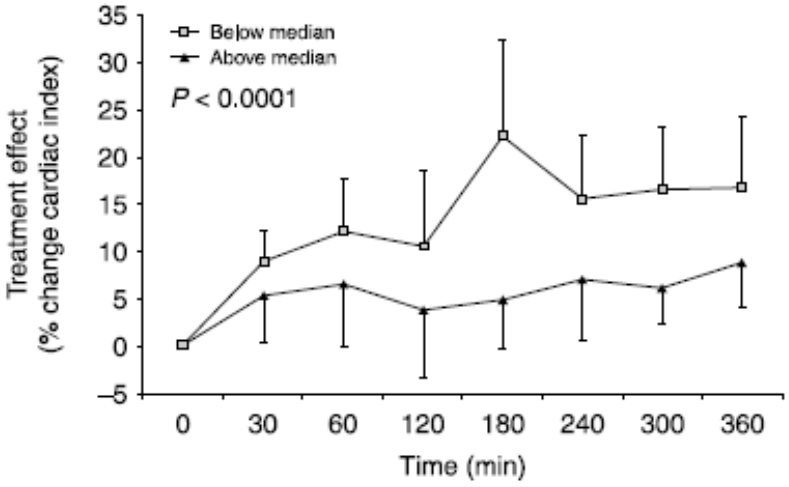

B
Eralpha -/- mice
Diameter (µm)
350
300
250
200
150
100
HF
NF
10 25 50 75 100 125

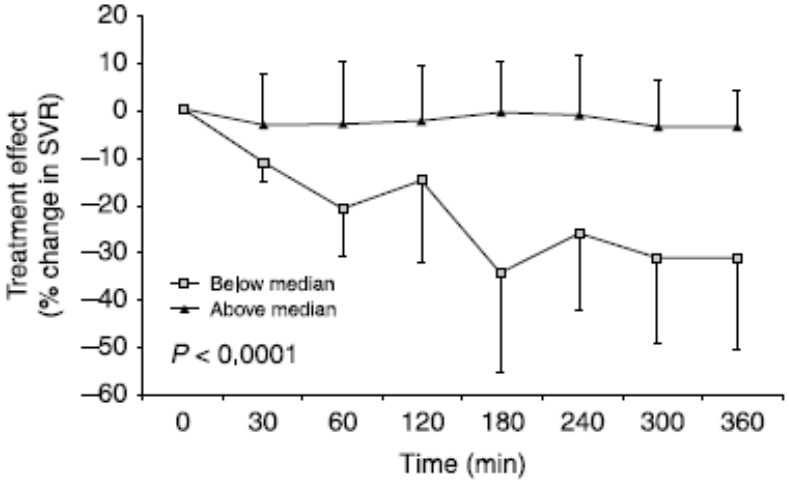

C
ER beta -/- mice
Diameter (µm)
350
300
250
200
150
100
HF
NF
*
10 25 50 75 100 125
Pressure (mmHg)

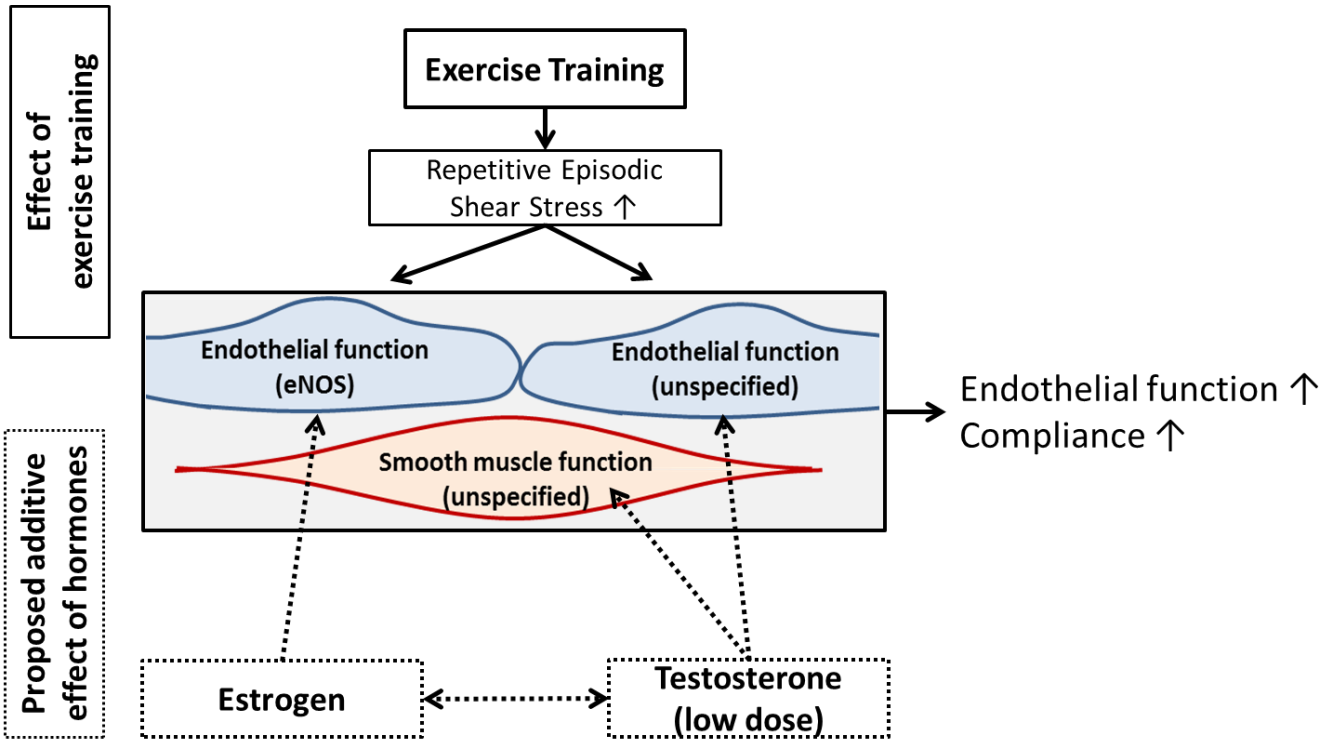

A. Early Intervention
B. Late Intervention
p = N.S.
p < .05
Coronary Artery Plaque Size (mm²)
0.40
0.30
0.20
0.10
0
Placebo
CEE
Baseline
Placebo
CEE
CEE+MPA


Plaque Size at Outcome (mm2)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
CEE
p=0.0001
p=0.33
p=0. 71
Baseline Tertile
Baseline PQ size (mm²)
Low
<0.12
Middle
0.12-0.60
High
>0.60
Plaque Burden


Bio-available testosterone (nmol/L)
Active
Placebo
Time (minutes)


Treatment effect
(% change cardiac index)
Below median
Above median
P < 0.0001
Time (min)


Treatment effect
(% change in SVR)
Below median
Above median
P < 0,0001
Time (min)


Effect of exercise training
Exercise Training
Repetitive Episodic Shear Stress ↑
Endothelial function (eNOS)
Endothelial function (unspecified)
Smooth muscle function (unspecified)
Endothelial function ↑
Compliance ↑
Proposed additive effect of hormones
Estrogen
Testosterone (low dose)