Sex differences in obesity-induced hypertension and vascular dysfunction: a protective role for estrogen in adipose tissue inflammation?*

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Submitted 18 May 2016; accepted in final form 7 August 2016

Taylor LE, Sullivan JC. Sex differences in obesity-induced hypertension and vascular dysfunction: a protective role for estrogen in adipose tissue inflammation? Am J Physiol Regul Integr Comp Physiol 311: R714–R720, 2016. First published August 10, 2016; doi:10.1152/ajpregu.00202.2016.—Obesity is a potent predictor of cardiovascular disease and associated risk factors, including hypertension. Systemic inflammation has been suggested by a number of studies to be an important link between excess adiposity and hypertension, yet the majority of the studies have been conducted exclusively in males. This is problematic since women represent ~53% of hypertensive cases and are more likely than men to be obese. There is a growing body of literature supporting a central role for immune cell activation in numerous experimental models of hypertension, and both the sex of the subject and the sex of the T cell have been shown to impact blood pressure (BP) responses to hypertensive stimuli. Moreover, sex steroid hormones play an important role in energy homeostasis, as well as in the regulation of immune responses; estrogen, in particular, has a well-known impact on both cardiovascular and metabolic disorders. Therefore, the purpose of this review is to examine whether sex or sex hormones regulate the role of the immune system in the development of hypertension and related vascular dysfunction in response to metabolic changes and stimuli, including a high-fat diet.

* Based on a presentation from the APS conference "Cardiovascular, Renal, and Metabolic Diseases: Physiology and Gender," November 17–20, 2016, in Annapolis, MD.

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Experimental Evidence: Sex Differences in Obesity- and HFD-Induced Hypertension

Although there are numerous studies that have examined the impact of high-fat feeding on BP in males and a handful of studies examining the impact of a HFD only in females, very few studies have directly compared the BP response to a HFD between the sexes. Gupta et al. (21) published the first study to examine sex differences in the development of obesity-associated hypertension in 2012. They found that treatment of male and female C57BL/6 mice with a HFD for 16 wk results in greater increases in body weight and fat mass in female mice relative to age-matched mice fed a low-fat diet compared with males. Despite this, male mice exhibit greater increases in BP on a HFD compared with females; BP was not significantly different between female mice fed a high-fat or a low-fat diet. Even in the absence of changes in BP, a HFD induces sex-specific alterations in additional cardiovascular parameters. HFD-induces greater increases in left ventricular mass, inner diameter, and wall thickness, as well as greater decreases in fractional shortening in male mice lacking eNOS vs. eNOS-deficient females (13). Consistent with this report, female C57BL/6 mice maintain better glycemic control in response to a HFD compared with males, while male mice exhibit greater diastolic dysfunction and hypertrophy (14).

Despite consistent reporting of sex differences in the impact of high-fat feeding on cardiovascular health, there are limited studies examining the molecular mechanisms responsible, although the available data implicate a sex difference in the balance of the RAS. Male C57BL/6 mice exhibit ANG II-dependent increases in BP with high-fat feeding (21), while greater angiotensin-converting enzyme (ACE)2 activation in adipose tissue in females protects against obesity-induced hypertension (60). Interestingly, the RAS has also been linked to inflammation. T cells are required for male mice to develop a robust increase in BP to ANG II (17), while ANG (1–7) has been suggested to be anti-inflammatory (43). Our laboratory has shown that female spontaneously hypertensive rats have more anti-inflammatory T regulatory cells (Tregs) and cytokines compared with males, as well as greater levels of ANG (1–7), both of which have been linked to lower BP in females (52, 56). On the basis of the central role of immune cell activation in both obesity and hypertension, taken together with sex differences in T cells, the remainder of this review will examine immune mechanisms, which may contribute to sex differences in HFD-induced hypertension.

Adipose Tissue: a Rich Source of Cytokines

Adipose tissue has the potential to play a pivotal role in promoting the chronic low-grade inflammation that is a hallmark of both obesity and hypertension. Adipose tissue is not merely a passive reservoir for lipid storage, but an active endocrine organ that secretes hormones and cytokines that have a direct influence on metabolism and energy homeostasis (37). Immune cells are recruited into adipose tissue in obesity leading to local inflammation, and several proinflammatory factors, including TNF-α, IL-1, IL-6, and C-reactive protein (CRP), are released by adipose tissue. Not all factors released from adipose tissue, however, are pathogenic; for example, adiponectin is also released from adipose tissue. Adiponectin levels are decreased in genetic and diet-induced experimental models of obesity, and decreases in adiponectin have been linked to insulin resistance and cardiovascular disease (64).

Many of these same factors have been linked to increases in BP, and some exhibit sex differences in response to high-fat feeding. A HFD results in greater increases in liver TNF-α mRNA in male C57BL/6 mice compared with females (17), and TNF-α has been linked to increases in BP in male and female experimental animals (15, 58). Therefore, although it is intriguing to speculate that sex differences in HFD-induced increases in BP are mediated by TNF-α, studies need to determine whether the sex difference observed in liver TNF-α is due to the HFD or known sex differences in BP. High-fat feeding also results in significantly greater circulating IL-6 levels in male C57BL/6 mice vs. female mice. Moreover, adipose tissue-specific knockout of IL-6 results in a decrease in body weight gain in response to a HFD only in female mice (38); in contrast, muscle-specific knockout of IL-6 results in an attenuated weight gain in males, while female IL-6 KO mice gained more weight compared with control littermates (16). Together, these studies suggest that tissue-specific effects may underlie the cardiovascular phenotype following a HFD in males vs. females.

Sex differences in plasma adiponectin levels have also been reported in both humans and rodents with females generally having higher levels than males (4, 20), and plasma adiponectin levels have been shown to be decreased in hypertension and obesity (61). However, there is not a clear consensus in the literature as to whether or not this sex difference is maintained following HFD or in obesity. Circulating adiponectin levels have been reported to increase only in male Wistar rats following 14 wk of HFD (55), decrease only in female Wistar rats following 26 wk of HFD (1), decrease in both male and female Wistar rats, although females exhibited a greater decrease than males, with 15 days of HFD (47), or decrease only in male C57BL/6J following 20 wk of HFD (14). These studies suggest that the duration of high-fat feeding may be an important determinant of the impact of HFD on adiponectin in both sexes, but more studies are needed both to resolve the impact of sex on HFD-induced alterations in adiponectin, as well as define the relationship between changes in adiponectin and BP. Regardless, higher levels of adiponectin have been linked to lower BP. Obese male KKAY mice exhibit reduced plasma adiponectin levels and a concomitant elevation in BP, which is reversed following administration of adiponectin, suggesting a protective role for adiponectin in obesity-induced hypertension in males (42). With regard to sex, female Sprague-Dawley rats exhibit blunted BP responses to salt, and L-NAME-induced increases in BP relative to males, and plasma adiponectin levels were 65% higher in females than in males at baseline. Moreover, injection of testosterone reduced plasma adiponectin levels and reversed the blunted BP responses in females, leading authors to conclude that higher levels of adiponectin were critical in the ability of the female to maintain a lower BP (26). Therefore, greater adiponectin in females following a HFD may be a critical component of the lower BP observed in response to high-fat feeding vs. males.
Perivascular adipocytes exhibit a proinflammatory phenotype in obesity or as a result of high-fat feeding. Specific ectopic fat depots have been implicated in the pathogenesis of CVD, including perivascular adipose tissue (PVAT), which surrounds most systemic blood vessels (6). PVAT directly influences vascular function via secretion of several bioactive substances; and under physiological conditions, PVAT exerts a net vasodilatory effect (54). In contrast, in pathological states, PVAT becomes dysfunctional and likely contributes to disease progression. PVAT impairs relaxation in both conduit arteries and small arterioles of HFD-fed male Wistar rats, which also exhibit a thicker aortic tunica media, indicating pathological vascular remodeling (34). These data strongly support the notion that PVAT contributes to vascular dysfunction. Although studies investigating sex differences in PVAT function are limited, obese women exhibit significantly higher epicardial fat and daytime BP compared with obese men, highlighting a potential role for ectopic fat depots in sex differences in BP control (50).

Inflammation has been implicated in the pathological changes that occur in PVAT during obesity, contributing to endothelial dysfunction. Perivascular adipocytes from human coronary arteries produce more proinflammatory cytokines compared with adipocytes from subcutaneous and visceral depots (19), and a HFD leads to greater increases in proinflammatory factors in perivascular adipocytes of male C57BL/6 mice compared with other fat depots (7). These studies suggest a particularly important role for PVAT in the induction of inflammation. It was recently shown that mammalian target of rapamycin (mTOR) may be the link between HFD, endothelial dysfunction, and inflammation. In response to a HFD, mTOR expression is increased in the aorta of male Wistar rats (34), and rictor, the essential regulatory protein of mammalian target of rapamycin complex 2 (mTORC2), is downregulated in thoracic PVAT leading to increased expression of proinflammatory genes concomitant with impairment in PVAT-mediated vasodilation (2). mTOR signaling is also associated with sex-dependent changes in CVD. Following inhibition of mTOR with rapamycin, DOCA-salt induced left ventricular hypertrophy is attenuated in male C57BL/6 mice but worsened in females, suggesting sex-specific effects of mTOR signaling (22). Moreover, while female C567BL/6 mice have higher intrinsic levels of mTORC1 and mTORC2 vs. males, mTORC activity is increased in males and decreased in females following rapamycin treatment, which likely contributes to the sex differences in cardiac remodeling (22). These studies support an important role for mTORC signaling in the development of CVD in females raising the possibility that differential effects of HFD on mTOR could underlie sex differences in inflammation following a HFD and explain how females can have greater increases in fat deposition vs. males yet a milder CVD phenotype. More studies are needed to investigate how components of the mTOR pathway are differentially regulated in response to HFD and their impact on inflammatory status and overall vascular function in both males and females.

Adipose Tissue: a Rich Source of Immune Cells

The innate and adaptive arms of the immune system play significant roles in the development of hypertension and related end-organ damage (48). In particular, both macrophages and T cells accumulate in the kidney and vasculature in numerous cardiovascular disorders; recent studies have shown significant immune cell infiltration into the adventitia and PVAT (48). As a result, adipose tissue is now recognized as a target organ for these immune cells in the context of obesity and related complications.

Macrophages. Macrophages are key players in innate immunity and protect the body either by phagocytosing infected cells or killing infected cells via cytokine release. The local tissue environment can polarize macrophages to either the proinflammatory M1 (classically activated) or anti-inflammatory M2 (alternatively activated) phenotype. M1 macrophages release proinflammatory cytokines and reactive oxygen species that worsen CVD, while M2 macrophages secrete anti-inflammatory cytokines and tissue repair proteins to offer cardiovascular protection (63). Thus, the phenotype of macrophages infiltrating different target organs can determine disease outcome.

Monocyte colony-stimulating factor (MCF)-1-deficient male mice lack functional macrophages and resist elevations in BP and vascular remodeling following ANG II or DOCA-salt treatment, supporting a critical role for macrophages in BP control (11, 29). HFD-induced obesity (20 wk of high-fat feeding) is also accompanied by an accumulation of macrophages in visceral adipose tissue in male C57BL/6 mice (33), and studies have previously established that 16 wk of HFD increases BP in this mouse strain (21). Macrophages in the adipose tissue of obese male mice also exhibit greater expression of proinflammatory markers of the M1 phenotype, and this was linked to insulin resistance, while adipose tissue from lean male mice express more anti-inflammatory markers of the M2 phenotype (33). Thus, obesity not only increases macrophage infiltration into adipose tissue but also induces a phenotypic switch in macrophage polarization in male mice to favor a more proinflammatory M1 phenotype (33). While much less is known in females, female C57BL/6 mice have fewer numbers of M1 macrophages in adipose tissue vs. males (45), which may help protect against features of metabolic syndrome. Studies are needed to directly assess the impact of HFD on macrophage infiltration and macrophage polarization in males vs. females and determine the relative contribution of macrophages to HFD-induced cardiovascular complications in both sexes.

T cells. T cells are key players in adaptive immunity (for a full review on T cells, see Ref. 32). Although there has been interest in the role of the immune system on BP and CVD for decades, the recent focus on T cells in BP control was sparked by studies in recombinase activating gene (RAG)-1-deficient mice, which lack functional T and B cells, in the last 10 years. Male RAG-1-deficient mice exhibit blunted hypertensive responses to ANG II and greater endothelium-dependent vasodilation compared with wild-type (WT) male mice, and adoptive transfer of T cells from male WT restores ANG II-induced hypertension and endothelial dysfunction (23). Interestingly, adoptive transfer of T cells from female WT mice to RAG-1-deficient male mice does not result in increases in ANG II-induced hypertension, suggesting that the sex of the T cell itself contributes to sex differences observed in hypertension (25).

Male RAG-1-deficient mice also gain more weight with greater visceral adiposity compared with WT mice on a HFD.
Sex Hormones: Do Female Sex Hormones Protect Against HFD-Induced Inflammation?

The cardiovascular benefits of female sex hormones have been well documented in both humans and experimental animals. Premenopausal women are generally considered protected from hypertension and CVD relative to age-matched men, and this protection is lost following the onset of menopause. A protective role for female sex hormones is further supported by experimental studies employing surgical removal of the ovaries; indeed, ovariectomy has been shown to promote hypertension in animal models of diet-induced hypertension. Male DSS rats exhibit greater increases in BP in response to salt compared with female DSS rats, and OVX results in salt-induced increases in BP comparable to the levels in males. Interestingly, even when maintained on a low-salt diet (0.15% NaCl), OVX results in the development of spontaneous hypertension in female DSS over time, indicating that female sex hormones protect DSS against both salt-dependent and salt-independent hypertension. Studies by our laboratory have confirmed that OVX results in a pronounced exacerbation of high-salt (4.0% NaCl)-induced increases in BP in female DSS rats (5). OVX also exacerbates obesity-induced increases in BP in female mice (21), and 17-β estradiol supplementation attenuates this increase in BP (60), providing direct support for a protective role for female sex hormones against diet-induced hypertension.

Estrogens tend to reduce fat intake and increase energy expenditure to limit body fat accumulation, and, hence, represent an important link to obesity-induced CVD (35). Thus, not surprisingly, ovariectomy leads to greater increases in food intake and body weight gain in response to a HFD in female B6D2 mice vs. mice on a normal-fat diet (31). Moreover, ovariectomized mice receiving cyclical 17-β estradiol supplementation gain significantly less weight than ovary-intact females due to reduced caloric intake. Ovariectomy may also lead to PVAT dysfunction; ovariectomy of female Sprague-Dawley rats attenuates the vasodilatory effects of PVAT compared with sham rats (59). On the basis of the wide range of physiological and biochemical actions of estrogens, it is unlikely that the only mechanism by which estrogen modulates cardiovascular responses to a HFD is via regulation of metabolic homeostasis. Indeed, although 17-β estradiol administration attenuates HFD-induced increases in body weight in both ovariectomized WT and ACE2-deficient mice, BP is reduced only in the WT mice, indicating a key role for ACE2 and the RAS in modulating the BP-protective effects of female sex hormones (60). 17-β Estradiol-mediated decreases in HFD-induced hypertension were accompanied by an increase in adipose tissue, but not renal, ACE2 activity, suggesting a selective effect of 17-β estradiol on adipose tissue is mediating the protective effects of the RAS. Furthermore, 17-β estradiol supplementation had no effect in male mice (60), reflecting a unique role for 17-β estradiol to modulate metabolic and cardiovascular responses to HFD in the female. The impact of male sex hormones on HFD or obesity-induced hypertension has not been examined to the best of our knowledge; however, orchidectomy decreases both blood pressure (53) and cardiac ACE2 (8) activity in male SHR, suggesting that the prohypertensive effects of male sex hormones could contribute to greater increases in BP in response to a HFD in males compared with females. More studies are needed to address this hypothesis.

Estrogens are also a determining factor in chronic inflammation, as demonstrated by the prevalence of autoimmune disorders among women and the variability in inflammatory status during the menstrual cycle, pregnancy, and menopause. Moreover, estrogen receptors (ER), including ERα, ERβ, and GPR30, are found on the surface of immune cells and can, thus, modulate the immune response (3, 27). The immunosuppressive activity of estrogens has been well established in several models of inflammatory disease, and, in general, 17-β estradiol inhibits proinflammatory Th17 and M1 macrophage cell differentiation and enhances Treg and M2 macrophage expansion (3, 27). Thus, 17-β estradiol signaling may play a key role in promoting anti-inflammatory immune cells to protect against hypertension and vascular dysfunction.

In support of this hypothesis, ovariectomized female C57BL/6 mice on a HFD exhibit increases in adipose tissue macrophages, and this increase was significantly attenuated following 17-β estradiol treatment, supporting a protective role for estrogens against HFD-induced inflammation (49). Consistent with this finding, high-fat feeding alone leads to a reduction in ERα expression in visceral adipose tissue, but not in muscle, of female Sprague-Dawley rats, and this occurs concomitant with activation of common stress kinases involved in inflammatory responses (18). In addition, knockdown of ERα in visceral adipose tissue results in enlargement of adipocytes and increases in macrophage expression in fat pads in female mice, and male and female mice lacking ERα in adipocytes...
exhibit increases in adipose tissue inflammation, suggesting that adipose tissue ERα protects against inflammation in both sexes (9).

**Clinical Outlook**

Several population and clinical studies have established an association between hypertension and obesity. The AT-TICA study was designed to examine the 5-year incidence of hypertension among prehypertensive, cardiovascular, disease-free adult men and women (46). One of the primary findings of the study was that people who developed hypertension within the 5-yr period and were less likely to adhere to a Mediterranean type of diet had higher anthropometric indices and were two times more likely to be obese. Increases in waist and hip circumference and BMI were also associated with higher risks of hypertension, with abdominal obesity being associated with a 2.09-times higher risk of hypertension, irrespective of sex.

CRP has been shown in numerous studies to be a major predictor of hypertension and CVD risk, and CRP stimulates monocytes to release proinflammatory cytokines (12). Moreover, the increased hypertension risk with obesity in the Multi-Ethnic Study of Atherosclerosis study was associated with higher serum levels of CRP (30), and in a study of African-American men and women, it was found that although women had higher mean BMI than men, they had greater levels of CRP (30 < 0.0001) and circulating plasma adiponectin (P < 0.0001) (10). Higher CRP levels observed in women vs. men can be correlated to accumulation of total body fat (28), yet the relationship between increases in visceral adiposity and hypertension is stronger in boys than in girls (44), supporting experimental studies of a sex/gender difference in the impact of a HFD on cardiovascula health. Together, these studies suggest that inflammatory mediators may contribute to both early and late increases in BP, with changes in body weight; however, additional studies are needed to define the interrelationship among obesity, inflammation, and hypertension clinically in both men and women.

**Perspectives and Significance**

The intimate contact of PVAT to the blood vessel wall allows it to directly influence vascular function and obesity, leading to inflammation, which can contribute to hypertension and end-organ damage. However, the molecular mechanisms underlying PVAT-mediated vascular dysfunction remain in question. This article sought to highlight the role of the immune system and inflammation as possible mechanisms contributing to HFD and obesity-related hypertension, as well as PVAT-mediated vascular dysfunction in both sexes. Studies exploring a mechanistic link between female sex hormones and immune cell activation in HFD-induced hypertension and vascular dysfunction are lacking. However, with the current knowledge, it is likely that in response to a HFD, there is a downregulation of ER expression on immune cells, promoting an overall proinflammatory immune response that favors hypertension and vascular dysfunction in females.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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**AUTHOR CONTRIBUTIONS**

L.E.T. and J.C.S. drafted manuscript; L.E.T. and J.C.S. edited and revised manuscript; L.E.T. and J.C.S. approved final version of manuscript.
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