Sex and Gender Differences in Cardiovascular, Renal, and Metabolic Diseases

Sexually dimorphic myeloid inflammatory and metabolic responses to diet-induced obesity

C. Griffin, N. Lanzetta, L. Eter, and K. Singer

Division of Pediatric Endocrinology, Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, Michigan

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Griffin C, Lanzetta N, Eter L, Singer K. Sexually dimorphic myeloid inflammatory and metabolic responses to diet-induced obesity. Am J Physiol Regul Integr Comp Physiol 311: R211–R216, 2016. First published June 1, 2016; doi:10.1152/ajpregu.00136.2016.—It is well known in clinical and animal studies that women and men have different disease risk as well as different disease physiology. Women of reproductive age are protected from metabolic and cardiovascular disease compared with postmenopausal women and men. Most murine studies are skewed toward the use of male mice to study obesity-induced metabolic dysfunction because of similar protection in female mice. We have investigated dietary obesity in a mouse model and have directly compared inflammatory responses in males and females. In this review we will summarize what is known about sex differences in diet-induced inflammation and will summarize our data on this topic. It is clear that sex differences in high-fat diet-induced inflammatory activation are due to cell intrinsic differences in hematopoietic responses to obesogenic cues, but further research is needed to understand what leads to sexually dimorphic responses.

diabetes; high-fat diet; myelopoisis; obesity; sexual dimorphism

Global obesity rates have risen drastically in the past several decades with around one in every three individuals currently categorized as obese (34). The overall incidence of obesity-related diseases such as diabetes and cardiovascular disease (CVD) also continues to rise as a result (3a). Obesity manifests as the result of an imbalance of caloric intake and energy expenditure. A major contributing factor to the increase in obesity rates is an increase in the consumption of calorie-dense foods rich in saturated fatty acids (4). With increased consumption of high-fat foods, individuals accrue body fat and thus have an elevated risk of developing obesity-related diseases. In this brief review we will emphasize the effects of diet-induced obesity, focusing primarily on sexually dimorphic responses of high-fat diet (HFD) priming of the immune system.

An individual’s response to HFD is dependent on several factors including sex, age, and ethnicity. What has become increasingly striking is that there is a clear sexual dimorphism in obesity and diabetes rates. While obesity rates are higher in women (34), men have higher rates of cardiovascular disease (CVD) and Type 2 diabetes (30, 36), suggesting that females are protected from the adverse effects of obesity (30). This is of particular importance because when investigating diabetes and CVD, many preclinical studies have been performed in males alone, leaving gaps in our knowledge of sexually dimorphic responses to obesity (45). Therefore, guidelines and therapies are being created based on investigations in males but are being implemented in men and women (13).

It is important to investigate males and females to understand the contributing factors to these sex-specific differences. Previous studies have focused on altered hormone environments, anatomical fat distribution (17, 19), and energy expenditure differences. Women have been found to have 10% higher total body fat content compared with males of the same body mass index (BMI) (15). This dimorphism is especially profound in poor socioeconomic conditions, whereas richer environments show a smaller variance in adiposity between the sexes (11). This suggests that estrogen largely influences fat accumulation regardless of socioeconomic status (11). Additionally, when adiposity matched, females display a greater volume of subcutaneous fat than males, whereas males have a greater volume of intra-abdominal or visceral fat (5, 9). Women are also known to have higher energy expenditure rates (47, 49). Limited data exist explaining this, but they indicate increases in brown adipose tissue (BAT) in females (17), and recent studies demonstrate the role of estrogen in sex differences in muscle metabolism (40). It has also been found that estradiol may influence the brain to decrease food intake and stimulate voluntary exercise, independent of its metabolic effects (51).
The prevalence of metabolic syndrome increases with age as women face higher BMIs (1), demonstrating a loss of inherent protection. One factor that contributes to this is menopause. Clinically, during menopause there is a shift in the circulating estradiol levels and an increase in the ratio of androgens that places women at increased risk for CVD and Type 2 diabetes (36, 42). Specifically, low levels of sex hormone binding globulin (SHBG), high free androgen levels, and low estradiol levels have been implicated in the CVD risk in perimenopausal women (48). In model mice, using ovarian failure to mimic the onset of menopause corresponds with an increase in insulin resistance (42). In this mouse model, the onset of insulin resistance was prevented following ovarian failure using estrogen replacement (17-β estradiol) therapy, thus further proving the importance of sex hormones on metabolic disease manifestation (42).

While sex hormones are a logical explanation for male and female differential responses to obesity, the clinical studies that have used estrogen replacement therapies have not been successful in preventing cardiovascular diseases (26). Thus further exploration in novel areas of investigation is required to close the gaps in our understanding of what links obesity to diseases in males and females.

**Obesity is Associated With Chronic Inflammation**

Inflammation may be either acute or chronic in nature, with the former having a protective role in the adipose tissue. In obesity-induced acute inflammation, leukocytes expand within the adipose tissue (55) to correct the physiological imbalance initiated by damaged adipocytes. If the stimulus is not resolved, chronic inflammation ensues, which has a more significant adverse effect on local and system tissue dysfunction (22). Though the mechanisms underlying the link between obesity and metabolic disorders are not completely understood, it is evident that they are associated with chronic low-grade inflammation (25). Obesity-induced inflammation, also known as meta-inflammation, has been implicated as a primary cause of metabolic disorders via activation of leukocytes, particularly of the myeloid lineage, in both human and murine models (20, 33). In obese individuals, several key inflammatory factors including pro-inflammatory cytokines such as IL-6, IL-1β, tumor necrosis factor-α (TNF-α), and chemokines such as MCP-1/CCR2 are elevated and associated with insulin resistance (32, 44). A major source of these systemic cytokines is the adipose tissue, specifically the visceral white adipose tissue (2). Within this depot, the adipose tissue macrophage (ATM) population is expanded under the influence of obesogenic diets, leading to systemic inflammation and disease. We have recently found evidence that fundamental changes occur within hematopoietic stem and progenitor cells (HSPCs) after HFD feeding, leading to an increase in production of macrophages. These changes in HSPCs lead to an increased production of granulocyte and macrophage progenitors and generate activated monocytes that are then recruited to become activated tissue macrophages (46).

Through this finding of hematopoietic stem cell (HSC) expansion and myeloid progenitor increases, we were able to conclude that dietary priming of hematopoietic progenitor cells leads to adipose tissue inflammation and that leukocyte production is enhanced through obesogenic signals (46). Other groups have also found that in murine models, obesity is a driver of proliferation and expansion of the bone marrow myeloid progenitors, with increasing monocytes and neutrophils in obese rodents compared with their lean counterparts (33). Overall, the drivers of this HSC activation in obese individuals remain unresolved.

**Sexually Dimorphic Responses in High-Fat Diet**

As previously stated, many of the above investigations that have been done to characterize inflammatory changes during diet-induced obesity have been performed in males. Female mice have been shown to be protected from insulin resistance and overall have dampened responses to HFDS in the laboratory. Several mechanisms have been investigated in an attempt to understand what protects females from the same metabolic impairments seen in males. The research in this area to date shows that male and female mice exhibit profound differences in anatomical adipose tissue distribution and expansion. Studies have shown that even when controlling for diet and other
environmental conditions, male mice show significantly greater expansion of total body mass including subcutaneous adipose tissue (SAT), visceral adipose tissue, and liver than their female counterparts (12, 14).

To understand mechanistic differences between males and females, investigations have focused on a variety of hormone models. Among these, it has been found that estrogen receptor α (ERα) is critical for protection against tissue inflammation observed in female mice (41).

Consistent with this, ovariectomized mice have a similar increased inflammation and insulin resistance (50). More recently, androgen receptor knockout models have shown similar benefits, with female knockout mice having a greater propensity toward developing metabolic disease (8). These studies have demonstrated that sex hormones are critical for the metabolic disease protection seen in females in response to obesity. While inflammation is so tightly linked to metabolic disease, there have been very few studies looking at changes in inflammation in female obese animals. Of the studies that have assessed metainflammation, an increase in inflammatory cytokines (7) and a decrease in T regulatory cells in obese male mice have been identified (37). These studies have experienced difficulty in creating a perfect model, because when exposed to HFD for the same time interval, female mice tend to gain weight more slowly than males. Thus females typically weigh less at the time of data collection. Interestingly, even when females have an equal body weight, they have more adiposity but dampened cytokine gene expression and glucose impairment compared with males (29, 35). Although there is likely a direct link of estrogen on macrophage development (3), the effects of this in the context of diet-induced obesity have not been directly investigated.

Sexually Dimorphic Macrophage Responses to High-Fat Diet

The mechanism behind this sexually dimorphic variance remains mysterious, especially in terms of regulation of myeloid inflammation. To understand the different inflammatory responses to HFD, we looked at ATMs and HSC/bone marrow (BM) populations in male and female animals on HFD (47). Using a 60% lard-based HFD chow, both males and females were able to gain weight and adiposity, though males gained more body weight compared with females. While females gained weight, adiposity, and had adipocyte hypertrophy on HFD, they had normal glucose tolerance and lower insulin levels compared with male mice. When we next looked at adipose tissue inflammatory changes in males after 16 wk of HFD, there was a clear expansion of macrophages, specifically of the CD11c+ ATMs that formed CLS. Females also exhibited an expansion of macrophages but primarily of the CD11c− type. To understand the inflammatory environment created in the adipose tissue, we looked at the adipose tissue gene expression of inflammatory cytokines and saw that expression was reduced in females on HFD compared with males on HFD (47).

With our prior findings that obese males have expansion of HSCs and myeloid progenitors, we evaluated monocytes and hematopoietic progenitors in this model. We found that while females had normal myeloid progenitors at baseline, these cells did not expand with HFD as we saw in males. Examining the bone marrow ex vivo, we found that female BM produced less granulocyte and macrophage colonies than males after palmitic acid (saturated fatty acid) stimulation and produced lower cytokine responses to lipopolysaccharides (LPS) (47).
Given the intrinsic BM changes along with the concern for differential weight gain and energy expenditure in males versus females, we next performed a competitive BM transplant (BMT) where male and female bone marrow could be evaluated in either a male or female recipient animal. After BMT we challenged recipient animals to HFD and found that no matter the recipient sex, male BM cells responded to diet-induced obesity by producing more ATMs, specifically more CD11c⁺ ATMs. This suggests that there is a cell-intrinsic difference in hematopoietic responses to obesity between the sexes. It may also indicate that there is a permanent, lifelong change within the HSC progenitor population after exposure to a HFD (47).

**DISCUSSION**

Over the last few decades there has been a greater understanding of the impact of diet-induced obesity on inflammation and insulin resistance, but there continue to be significant gaps in our knowledge on sexually dimorphic inflammatory responses. Through the use of male and female competitive BMTs we were able to conclude that there is an intrinsic sexual dimorphism in the bone marrow HSC and progenitor populations in response to diet-induced obesity, which is independent of differential weight gain and energy expenditure, though the driver of this dimorphism is still unclear (Fig. 1).

Research in this field has identified that sex hormones, adipocyte properties, and genetics are key drivers of male and female differences in obesity, diabetes, and cardiovascular disease. Estrogen plays a systemic role, improving energy balance through neuronal signals, pancreatic β cell survival, improved lipid metabolism, and insulin sensitivity within the liver and muscle (28). It has been shown that the effects of ERα occur via its activation in adipocytes especially in males. In the context of cardiovascular disease, myeloid-specific ERα deletion actually has been shown to induce insulin resistance and atherosclerosis (39). As was mentioned previously, ovariec-tomized mice also show an increase in adipose tissue inflammation and insulin resistance (50) likely via alterations in MCP1 and increases in reactive oxygen species (ROS) after ovariectomy (18).

Given that hormone replacement therapy has proven ineffective at decreasing the risk of metabolic and cardiovascular disease in obese patients, hormone independent changes have become an important area of work. In our studies there was an observable, hormone-independent, dimorphism in male and female myeloid cells based on ex vivo culture studies and competitive BMT results, finding that male myeloid cells are more activated and proinflammatory. This phenomenon of sexually dimorphic inflammatory responses has been under investigated, but studies have demonstrated lower systemic LPS responses in females (27). Contrary to this, Calippe et al. (3) showed that estradiol promotes proinflammatory cytokines via ERα activation on macrophages after ovariectomy. It is still unclear what role estrogen has directly on macrophage production and macrophage polarization, especially in specific clinical scenarios such as diet-induced obesity. In addition, whereas it was not in the scope of this current review, there is some data suggesting that testosterone supplementation may increase muscle mass and decrease fat mass in males with low levels of testosterone (57). Testosterone administration in female to male transsexuals alters adipose tissue distribution, with long-term administration instigating increased visceral fat and decreased subcutaneous fat deposits (6). Additionally, ATMs have been found to express the androgen receptor, potentially contributing to the metabolic effects of androgens in males (43).

**Perspectives and Significance**

It has been clear for centuries that there are sex differences in body composition and responses to HFD, but it has only recently become clear that there are inflammatory differences between the sexes regarding diet-induced inflammation. The findings in our studies and others emphasize that it is not simply hormones that directly affect insulin resistance, but rather, a combination of body composition, energy expenditure, appetite, hormone effects on insulin production, and inflammatory responses to diet that create the sexually dimorphic rates of obesity-related diseases. It is necessary to continue further investigating these differences to truly have a greater understanding of what leads to the clinical paradigm of sex differences in response to obesity.

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**AUTHOR CONTRIBUTIONS**

C.G. and K.S. interpreted results of experiments; C.G. and K.S. prepared manuscripts; K.S. analyzed data.

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C.G. and K.S. interpreted results of experiments; C.G. and K.S. prepared manuscripts; K.S. analyzed data.

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