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Sex differences in markers of metabolic syndrome and adipose tissue inflammation in obesity-prone, Osborne-Mendel and obesity-resistant, S5B/Pl rats

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ABSTRACT

The current study examined the role of sex differences in the development of risk factors associated with obesity and its comorbidities using models that differ in their susceptibility to develop obesity, obesity-resistant S5B/Pl (S5B) and obesity-prone Osborne-Mendel (OM) rats. Male and female rats were fed a low fat or high fat diet (HFD) and markers of metabolic syndrome (MetSyn) and expression of inflammatory cytokines/chemokines in visceral and subcutaneous adipose depots were measured. We hypothesized that male and female OM and S5B rats would exhibit differential responses to the consumption of HFD and that females, regardless of susceptibility to develop obesity, would display decreased obesity-related risk factors. Results suggested that consumption of HFD increased adiposity and fasting glucose levels in male OM and S5B rats, decreased circulating adiponectin levels in male S5B rats, and increased body weight and triglyceride levels in male OM rats. The consumption of HFD increased body weight and adiposity in female OM rats, not female S5B rats. Overall, female rats did not meet criteria for MetSyn, while male rats consuming HFD met criteria for MetSyn. Visceral and subcutaneous adipose tissue inflammation was higher in male rats. In visceral adipose tissue, HFD consumption differentially altered expression of cytokines in male and female S5B and OM rats. These findings suggest that resistance to obesity in males may be overridden by chronic consumption of HFD and lead to increased risk for development of obesity-related comorbidities, while female rats appear to be protected from the adverse effects of HFD consumption.

1. Introduction

Obesity is a chronic, multi-factorial metabolic disease characterized by excess adiposity [10,11,54] and an increased risk for the development of obesity-related comorbidities, such as cardiovascular disease (CVD) and type 2 diabetes (T2D) [17]. Approximately 42% of adults in the United States are considered obese, with women more likely to become extremely obese (BMI > 40) [27,36]. Individual differences in the susceptibility to develop obesity, differences in body composition and regional fat distribution and the consumption of energy dense foods contribute to the risk of developing obesity-related comorbidities [3,9,10,14,15,58–60]. However, few studies have investigated the role of sex differences in the susceptibility to develop obesity and the subsequent effects on adipose inflammation and risk factors associated with the development of cardiometabolic disease.

Regional distribution of adipose tissue between subcutaneous and visceral depots is predictive of health-related risks, with visceral adipose tissue (VAT) having the largest effect on metabolic dysfunction and leading to an increased risk for the development of CVD, T2D and metabolic syndrome (MetSyn), which is defined as a cluster of obesity-related metabolic abnormalities [6,16,22,29,30]. Visceral fat accumulation is associated with increased infiltration of macrophages and secretion of pro-inflammatory adipokines/cytokines, which contribute to the development of cardiometabolic disease [14,26,28,42,53,61,62,71,72].

Obesity-prone Osborne-Mendel rats (OM) and obesity-resistant S5B/Pl rats (S5B) are preclinical models used to study mechanisms that contribute to the individual susceptibility to develop obesity [3,18,23,32,48,52,59,60,64,65,67,74]. Inherent differences in visceral adipose inflammation, adipocyte size, expression of cardiovascular

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disease biomarkers, glucose regulation, and circulating adiponectin and lipids have recently been reported between male OM and S5B rats [59,60]. Consumption of high fat diet (HFD) in male OM, compared to male S5B rats, leads to a significant increase in body weight and visceral adiposity. However, consuming HFD significantly increases the expression of pro-inflammatory cytokine expression in epididymal fat depots in both strains and increases the number of crown-like structures in obesity-resistant S5B rats, suggesting that HFD consumption also increases the risk for obesity-related comorbidities in male S5B rats [60]. Few studies have investigated the sex differences in the susceptibility to develop obesity and its comorbidities in these strains [4,12,57,68,69].

Recently, an emphasis on examining sex differences in metabolic disorders has emerged. Epidemiological studies focused on examining sex differences in the susceptibility to develop obesity have shown that men are more likely to deposit VAT whereas women tend to accrue subcutaneous adipose tissue (SAT) [21,44,63,70]. Furthermore, females often have a larger fat to total mass ratio compared to males and sexual dimorphisms have been reported for circulating levels of the adipokines, leptin and adiponectin [8,13,20,34,37,43,50]. Despite having an overall lower fat mass and lower prevalence of extreme obesity, the prevalence of prediabetes/diabetes, CVD and insulin resistance is higher in men compared to women [31,46].

The goal of the current study was to determine the sex-specific response to HFD consumption on weight gain, adiposity, adipose tissue inflammation and markers of MetSyn in male and female OM and S5B rats. We hypothesized that male and female S5B and OM rats would exhibit differential responses to HFD consumption. More specifically, male rats would exhibit an increased prevalence of markers for MetSyn and adipose inflammation in comparison to female rats consuming the same diet, suggesting that male rats are at a higher risk for the development of obesity-related comorbidities. We further hypothesized that though females would have higher adiposity levels, they would be "metabolically healthy", as measured by decreased markers of MetSyn.

2. Materials and methods

2.1. Animals

Adult (10–11 weeks old) female and male obesity-prone Osborne-Mendel (OM) and obesity-resistant, S5B/Pl (S5B; bred in the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved Pennington Biomedical Research Center and LSU Health Sciences Center vivariums) were used in this study. Animals were individually housed on a 12-h light/dark cycle (lights on at 0700) with ad libitum access to food and water. All procedures were approved by the Pennington Biomedical Research Center and LSU Health Sciences Center Institutional Animal Care and Use Committees (IACUC).

2.2. Diet conditions

Rats were randomly assigned to diet condition and given access to either a pelleted HFD (60% kcal from fat, Research Diets, D12492, New Brunwick, NJ; n = 6 female rats/strain, n = 6 male rats/strain) or a pelleted LFD (10% kcal from fat, Research Diets, D12450B, New Brunwick, NJ, n = 6 female rats/strain, n = 6 male rats/strain) for 7 weeks. Body weight and estimated percent visceral adiposity ((VAT, gonadal weight (g) + retroperitoneal weight (g)/body weight (g)) × 100) was determined at sacrifice.

2.3. Fasting glucose

Following 6 weeks of ad libitum access to HFD or LFD, all animals underwent an overnight fast (16 h) for measurement of fasting glucose levels. Tail blood was used to determine circulating glucose levels and glucose was measured using a glucometer (Contour blood glucose monitoring system, Bayer Health Care, Mishawaka, Indiana, USA).

2.4. Blood processing and analyses

All rats were killed under 3% isoflurane anesthesia and blood (~3–5 mL) was collected immediately via cardiac puncture. Blood samples were centrifuged at 3000 rpm for 10 min at 4 °C. Serum was stored at −80 °C until analysis. Serum triglyceride (TRG) levels were measured using the Triglyceride Colorimetric Assay Kit (Cayman Chemical, Ann Arbor, MI). Serum total cholesterol levels were determined using Cholesterol Assay Kit (Abcam, Cambridge, MA). Serum levels of adiponectin were measured using a commercially available Rat Adiponectin ELISA kit (Abcam, Cambridge, MA). Assays were performed based on manufacturer's instructions.

2.5. Assessment of metabolic syndrome

Metabolic markers for MetSyn were measured and MetSyn was determined by the presence of three or more of the following criteria: a greater than 10% increase in body weight compared to LFD fed rats (same strain/sex), percentage of VAT significantly greater than LFD fed rats (same strain/sex), fasting glucose greater than 100 mg/dL, total cholesterol higher than 175 mg/dL, and adiponectin levels significantly lower than LFD fed rats (same strain/sex) [1,2,25,39].

2.6. Adipose tissue processing and cytokine/chemokine assessment

Gonadal, mesenteric and inguinal fat depots were dissected at time of sacrifice. Samples of 100 mg were collected, frozen in liquid nitrogen and stored at −80 °C until further processing. Protein was isolated from adipose tissue samples as previously described by Poret et al. [61]. Briefly, samples were incubated on ice in protein extraction buffer (T-PER, Thermo Scientific Rockford, IL) containing phosphatase and protease inhibitors followed by homogenization for 30 s or until a smooth suspension was achieved. Samples were then centrifuged at 14,000 rpm for 10 min at 4 °C. Sample homogenates were collected, and protein concentration was measured using a BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL). As previously described [61], a Rat Cytokine/Chemokine Magnetic Bead Panel-Immunology Multiplex Assay (Millipore Sigma, Billerica, MA) to measure the expression of 7 cytokines/chemokines: IL-10, IL-1α, IL-1β, IL-6, MIP-1α (also known as CCL3), MIP-2 and TNFα in adipose tissue homogenates. Cytokine/Chemokine expression was normalized to protein values for each sample.

2.7. Statistical analyses

All data were analyzed with a two-way ANOVA with sex and diet as factors. Strains were assessed separately. Bonferroni post-hoc tests were used to determine differences when a significant interaction was detected. A significance level of $p < .05$ was used for all tests.

3. Results

3.1. Sex differences in the effects of HFD consumption on visceral adiposity, body weight and markers of metabolic syndrome

Body weight and percent visceral adiposity were measured following 7 weeks of chronic HFD consumption in S5B and OM rats. In S5B rats, males weighed more than females ($F = 499.0$, $p < .001$; Fig. 1A). HFD consumption did not significantly alter body weight in male or female S5B rats. In OM rats, a significant diet x sex interaction on body weight was detected ($F = 4.5$, $p < .05$; Fig. 1B). OM males weighed more than OM female rats. HFD consumption increased body weight in both male and female OM rats compared to rats fed the LFD. In S5B rats, percent visceral adiposity was higher in the females ($F = 76.1$, $p < .001$; Fig. 1C) and HFD consumption increased adiposity i ($F = 5.5$, $p < .001$). Post-hoc analyses revealed that this effect was specific to male S5B rats ($p < .05$). Consumption of HFD increased visceral adiposity in both male and

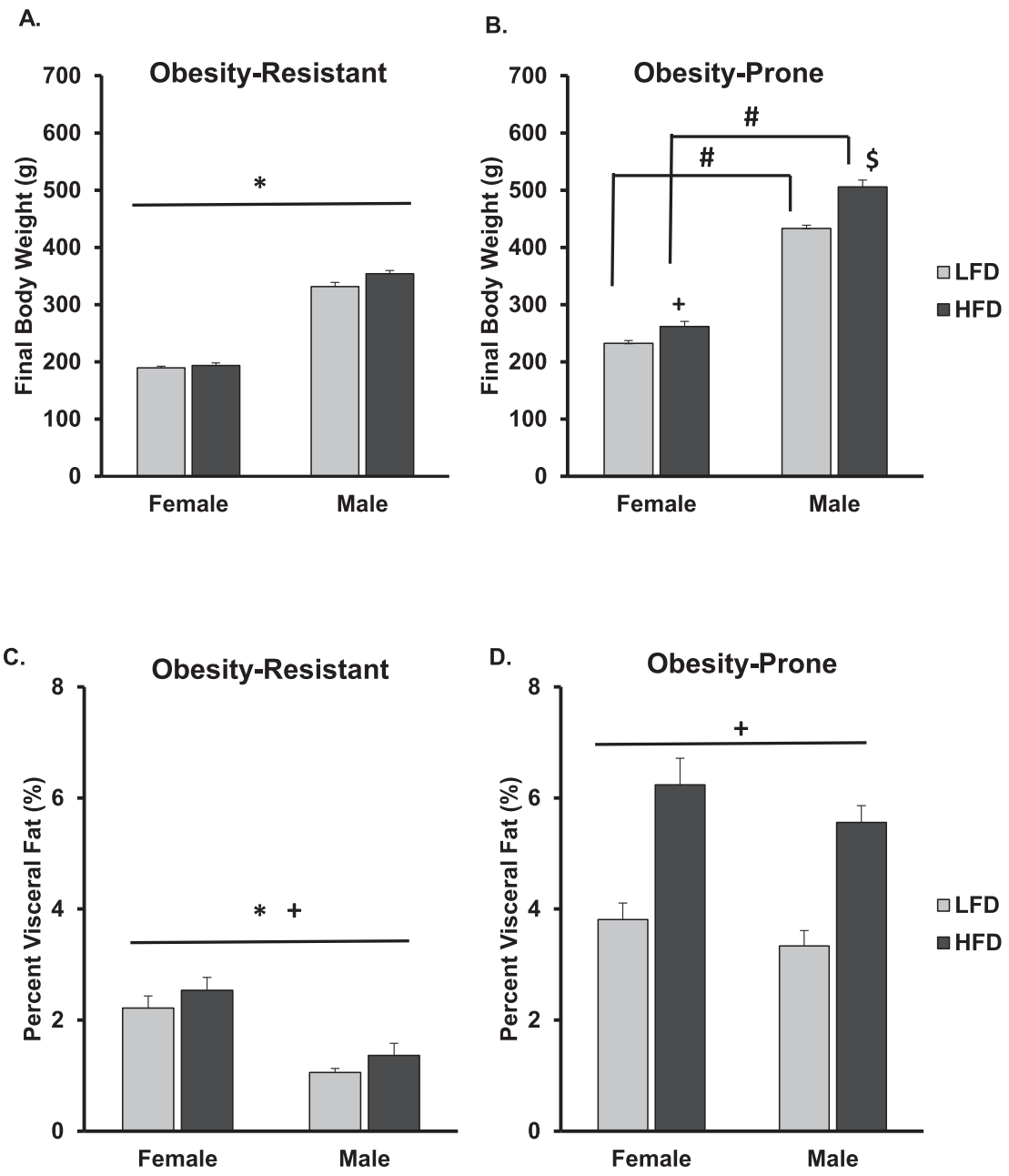

Fig. 1. Final body weight and visceral adiposity measurements in S5B and OM rats. **A.** Final body weight of male S5B rats was higher than females and not affected by HFD intake. **B.** Final body weight of male OM rats was higher than female OM rats and HFD increased body weight in OM rats. **C.** Visceral adiposity was higher in female S5B rats and HFD consumption increased visceral adiposity in S5B rats. **D.** In OM rats, visceral adiposity did not differ between male and female rats. HFD consumption increased visceral adiposity in OM rats. * $p < .05$ main effect of sex; # $p < .05$ male vs. female, same diet; +$p < .05$ main effect of diet; $ $p < .05$ LFD vs. HFD, same sex. Data shown as mean ± SEM.

female OM rats ($F = 42.5$, $p < .001$; Fig. 1D). A sex difference was not observed in OM rats.

Measures of glucose homeostasis were assessed following HFD or LFD consumption. In S5B rats, a significant diet × sex interaction was detected for fasting blood glucose levels ($F = 6.5$, $p < .02$; Fig. 2A). Female S5B rats had lower fasting blood glucose levels than male S5B rats fed the same diet. A significant diet × sex interaction on fasting blood glucose level was also detected in OM rats ($F = 4.3$, $p = .05$; Fig. 2B). OM male rats consuming the HFD had higher fasting blood glucose levels than female OM rats fed HFD. A significant interaction between diet and sex on serum adiponectin, an adipokine involved in regulating glucose levels, was detected in S5B rats ($F = 28.0$, $p < .001$; Fig. 2C). Post-hoc analyses revealed that serum adiponectin levels were higher in female S5B rats compared to male S5B rats. HFD consumption increased adiponectin in S5B females and decreased adiponectin levels in S5B males. In OM rats, a main effect of sex on circulating adiponectin levels was detected. Overall, female OM rats had higher levels of adiponectin than OM males ($F = 711.0$, $p < .001$; Fig. 2D).

Measures of circulating lipids were also assessed following consumption of HFD. Serum total cholesterol ($F = 66.7$, $p < .001$; Fig. 3A) was higher in S5B females and serum triglyceride ($F = 17.34$, $p < .001$; Fig. 3C) was higher in S5B males compared to S5B females. HFD consumption did not alter total cholesterol or serum triglycerides in S5B males or females. OM male rats had higher total cholesterol levels than OM female rats ($F = 20.2$, $p < .001$; Fig. 3B). An interaction between sex and diet was detected for triglyceride levels in OM rats ($F = 6.0$, $p < .05$;

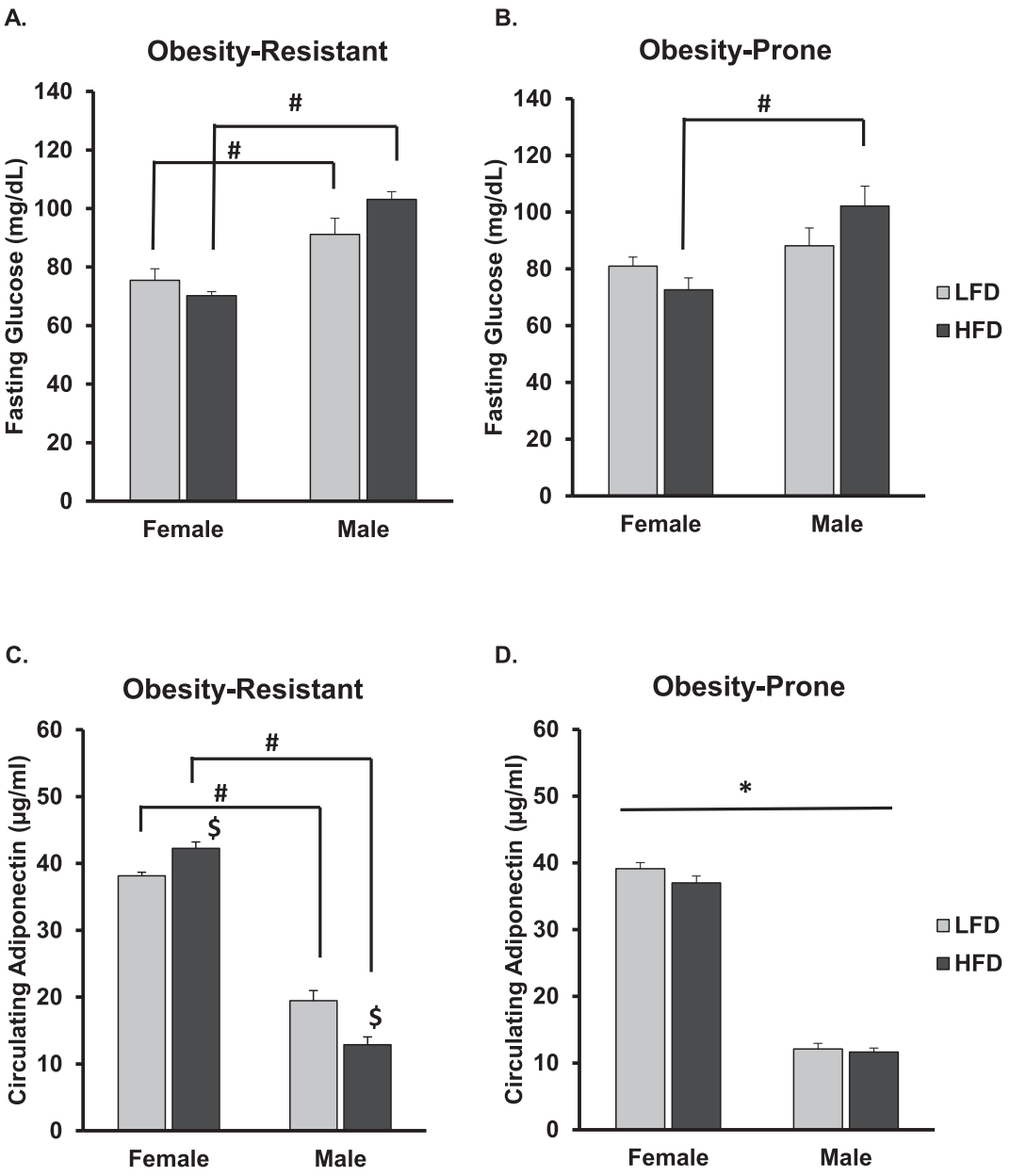

Fig. 2. Fasting blood glucose and circulating serum adiponectin levels in S5B and OM rats. **A**. Male S5B rats had higher fasting blood glucose levels compared to female S5B rats fed the same diet. **B**. HFD consumption increased fasting blood glucose levels in male OM rats compared to female OM rats consuming HFD. **C**. Female S5B rats had higher levels of serum adiponectin compared to male S5B rats. HFD consumption increased adiponectin levels in female S5B rats but decreased levels in male S5B rats. **D**. OM females had higher serum adiponectin levels compared to male OM rats. HFD intake did not alter adiponectin levels in OM rats. * $p < .05$ main effect of sex; # $p < .05$ male vs. female, same diet; $ $p < .05$ LFD vs. HFD, same sex. Data shown as mean ± SEM.

Fig. 3D). Post-hoc analyses revealed that male OM rats, fed either HFD or LFD, had higher serum triglyceride levels than female OM rats.

3.2. Determination of metabolic syndrome

Based on variables measured in the current study, three of six criteria were required for the determination of having MetSyn. Male S5B and OM rats consuming HFD met the criteria for MetSyn (Table 1). Female rats and male S5B and OM rats consuming LFD failed to meet criteria for MetSyn.

3.3. Sex differences in the effects of HFD consumption on adipose tissue inflammation

Protein expression of 7 cytokines/chemokines were assessed in gonadal, inguinal and mesenteric adipose tissue. In the gonadal fat of female S5B and OM rats, expression of 3 of 7 cytokines/chemokines was detected (IL-10, IL-1β, IL-6) while 4 cytokines/chemokines were not detected (IL-1α, TNF-α, G-CSF, MIP-1α, MIP-2). In the inguinal fat of females, 2 of 7 cytokine/chemokines measured were detectable (IL-1β, IL-10) and in mesenteric fat, 2 of 7 cytokine/chemokines were detectable (IL-10, MIP-1α). Therefore, statistical analyses were only performed on cytokines/chemokines that were detectable in both sexes for each fat depot.

In gonadal fat, a visceral fat depot, a significant interaction between sex and diet was detected for expression of IL-10 in S5B rats ($F = 7.5$, $p < .02$; Fig. 4A) and OM rats ($F = 11.6$, $p < .01$; Fig. 4B). Male S5B and OM rats fed HFD had higher gonadal expression of the anti-inflammatory cytokine, IL-10, compared to female S5B and OM rats fed HFD. Consumption of HFD reduced gonadal IL-10 expression in

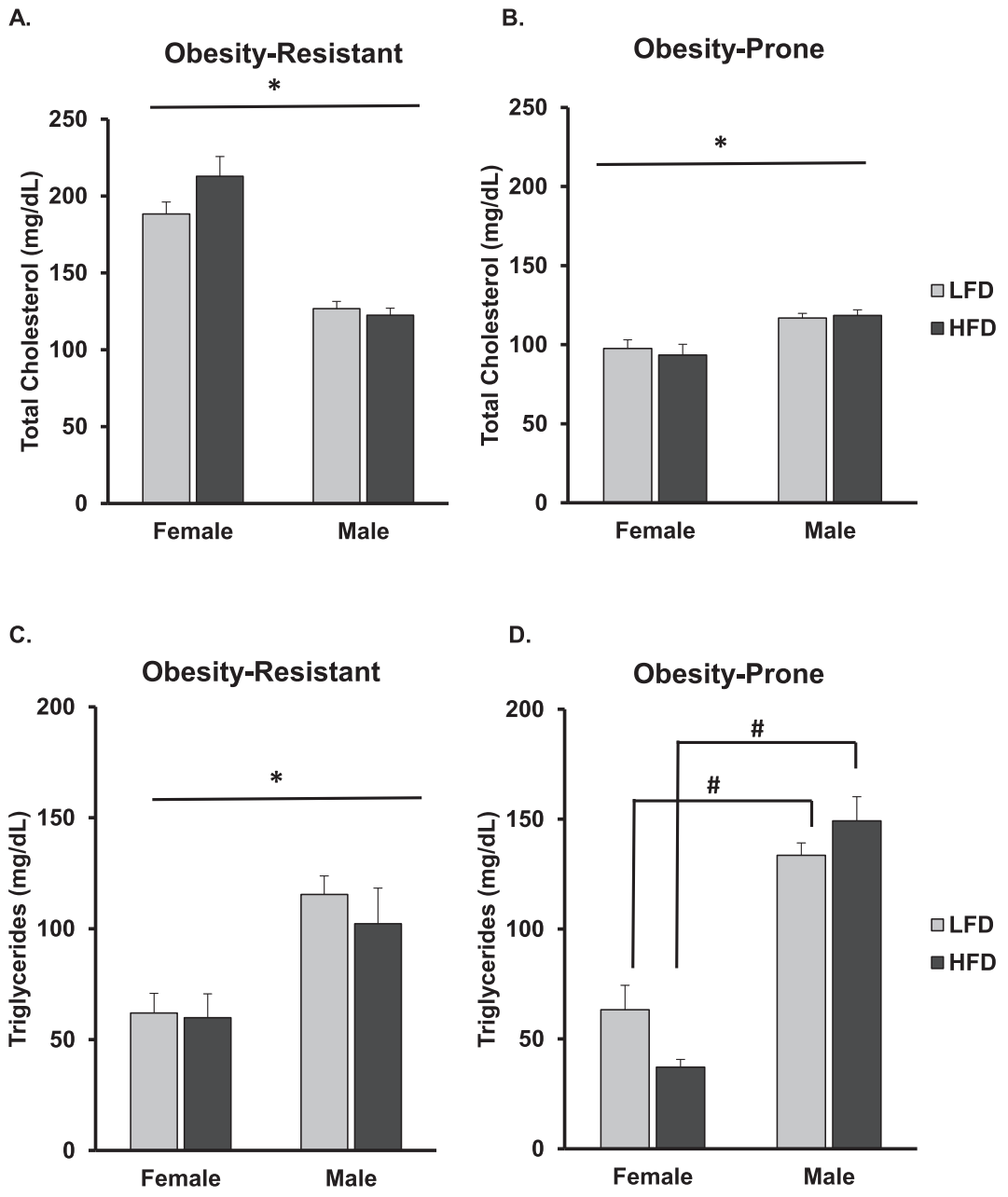

Fig. 3. Total cholesterol and triglycerides were measured in male and female S5B and OM rats. **A**. In S5B rats, female rats had higher circulating levels of total cholesterol than males. **B**. In OM rats, male rats had higher levels of total cholesterol than females. **C** Triglyceride levels were higher in male S5B rats, compared to females. **D**. Male OM rats had higher triglyceride levels than female OM rats. *p < .05 main effect of sex; # p < .05 male vs. female, same diet. Data shown as mean ± SEM.

female S5B rats. HFD consumption increased gonadal IL-10 expression in male OM rats, but not in female OM rats. A significant interaction between sex and diet was also detected for gonadal IL-1β expression in S5B rats ($F = 12.4$, $p < .01$; Fig. 4C) and OM rats ($F = 13.1$, $p < .01$; Fig. 4D). Expression of IL-1β, a pro-inflammatory cytokine, was higher in female S5B rats compared to male S5B rats consuming the LFD. HFD intake decreased IL-1β levels in female S5Bs and increased IL-1β levels in males. In OM rats, gonadal expression of IL-1β was higher in males and was significantly increased by consumption of HFD. A significant interaction between sex and diet was detected for gonadal expression of IL-6 in S5B rats ($F = 5.6$, $p < .05$; Fig. 4E). A main effect of sex was detected for IL-6 expression in OM rats ($F = 23.5$, $p < .001$; Fig. 4F). Gonadal expression of the pro-inflammatory cytokine IL-6 was higher in male S5B, compared to female S5B rats and was significantly increased by HFD consumption in males.

In mesenteric fat, a visceral fat depot, a significant main effect of diet ($F = 11.75$, $p < .01$) and sex ($F = 32.12$, $p < .0001$) on IL-10 expression was detected in S5B rats (Fig. 5A). Overall, male S5B rats expressed higher levels of IL-10 in mesenteric fat and HFD consumption reduced mesenteric IL-10 expression. A significant interaction between sex and diet was detected on mesenteric IL-10 expression in OM rats ($F = 11.09$, $p < .01$; Fig. 5B). HFD consumption reduced IL-10 expression in females. Female OM rats expressed higher levels of IL-10 than male OM rats fed LFD. A significant interaction between sex and diet on mesenteric expression of MIP-1α, a pro-inflammatory chemokine, in S5B rats was also detected ($F = 7.64$, $p < .02$; Fig. 5C). Female S5B rats expressed lower levels of MIP-1α than male S5B rats. Consumption of HFD decreased MIP-1α expression in S5B males. In OM rats, a main effect of

Table 1
Criteria for Metabolic Syndrome in Obesity-Resistant and Obesity-Prone male and female rats fed a HFD or LFD for 7 weeks. Three or more criteria must be met to be considered Metabolic Syndrome. ++ indicates that group meets indicated criteria.

	Obesity-resistant rats				Obesity-prone rats			
	Female		Male		Female		Male	
	LFD	HFD	LFD	HFD	LFD	HFD	LFD	HFD
>10% increase in BW from LFD group (same sex)						++		++
% VAT significantly > than LFD group (same sex)				++		++		++
Fasting Glucose > 100 mg/dL				++				++
Adiponectin significantly < LFD group (same sex)				++				
Total cholesterol > 175 mg/dL	++	++						
Triglyceride > 150 mg/dL								++
Met criteria for metabolic syndrome				++				++

sex on mesenteric expression of MIP-1α was detected ($F = 13.42$, $p < .01$). OM males expressed higher levels of MIP-1α than OM females.

In inguinal fat, a subcutaneous fat depot, a main effect of sex on IL-10 expression ($F = 26.2$, $p < .001$, Fig. 6A) and IL-1β expression ($F = 11.8$, $p < .01$, Fig. 6C) were detected in S5B rats. Males expressed more of the anti-inflammatory cytokine, IL-10 and the pro-inflammatory cytokine, IL-1β, than females. In OM rats, a significant main effect of sex was detected for IL-10 expression ($F = 8.04$, $p < .01$, Fig. 6B) and IL-1β expression ($F = 15.2$, $p < .001$, Fig. 6D). OM males expressed higher levels of IL-10 and IL-1β in inguinal fat depots. Consumption of HFD did not alter expression of these cytokines.

4. Discussion

Individual susceptibilities to develop obesity, regional fat distribution, adipose inflammation and the consumption of dietary fat contribute to the risk of developing obesity-related comorbidities including MetSyn, CVD and T2D [3,9,10,14,15,17,35,58–60]. The investigation of sex differences on the susceptibility to develop obesity and the subsequent development of cardiometabolic risk factors is limited. The current study investigated the effects of the consumption of HFD on the development of MetSyn, circulating lipids, glucose and adiponectin and adipose tissue inflammation in male and female rat models that differ in their propensity to develop obesity. Females often have a higher percentage of adipose tissue compared to males, however females have lower rates of prediabetes/diabetes, CVD and insulin resistance than males [8,13,20,31,34,37,43,46,47,50]. Therefore, we hypothesized that in OM and S5B rats, females would have higher adiposity levels, particularly when consuming the HFD, however, would have a lower risk for developing MetSyn and lower levels of visceral adipose inflammation.

Consumption of the HFD for 7 weeks did not significantly alter body weight in either male or female obesity-resistant S5B rats, though percent visceral fat was increased. In obesity-prone OM males and females, HFD intake increased body weight and visceral adiposity. In S5B rats, the visceral adiposity was higher in females, than males, however, in OM rats, adiposity levels were similar between males and females (Fig. 1). Difference in body weight between male and female rats fed LFD and HFD was calculated and an increase in body weight by more than 10% from the LFD group of the same sex and strain was a criterion for MetSyn. Obesity-resistant S5B rats consuming HFD had 2.1% and 6.8% higher body weights for females and males, respectively, consuming LFD. Obesity-prone rats consuming HFD had 12.6% and 16.7% higher body weights from females and males, respectively, consuming LFD, supporting the use of OM rats as a model of enhanced susceptibility to develop obesity. OM males and females met the body weight criterion for MetSyn (Table 1). In this study, percent visceral adiposity was significantly increased by HFD consumption in male S5B rats and male and female OM rats, therefore meeting criterion for MetSyn for these groups.

Circulating levels of glucose, adiponectin and lipids are markers of metabolic dysregulation and glucose and lipid levels are used clinically as risk factors for cardiometabolic disease. Overall, fasting glucose levels were higher in male S5B rats than female S5B rats and in male OM rats than female OM rats (Fig. 2). Fasting glucose levels are used clinically to signal the potential presence of prediabetes or diabetes. Fasting blood glucose levels in male S5B and male OM rats consuming the HFD were higher than 100 mg/dL, thereby meeting this criterion for MetSyn and supporting a sex difference in glucose regulation in both strains (Table 1). Adiponectin, an insulin-sensitizing hormone, is produced and secreted from adipocytes and has an inverse association with adiposity, so that lower levels of adiponectin are seen with higher levels of adiposity [5,43,49]. Additionally, adiponectin is a known modulator of many metabolic processes including regulation of glucose metabolism and energy metabolism [24,41,49], and lower levels of adiponectin are associated with several conditions such as T2D, coronary heart disease, hypertension and MetSyn and increased levels of several markers of inflammation [7,38,40,43,45,49,55,75]. A sex difference in circulating adiponectin levels has been reported and serum adiponectin levels in both female humans and rodents are higher compared to levels in male humans and rodents, which may be related to sex hormone levels and seems to be influenced by aging and fat depot distribution [8,13,20,34,43]. In the current study, higher levels of circulating adiponectin were found in female S5B and OM rats, compared to males. HFD consumption in S5B females increased levels of serum adiponectin, while decreasing adiponectin levels in male S5B rats (Fig. 2C). Sex differences in adiponectin levels could be a factor contributing to the higher rates of cardiometabolic disease in males compared to females. Though insulin sensitivity was not directly measured in this study, adipocytes from female rats have been shown to exhibit increased insulin sensitivity and glucose metabolism compared to adipocytes from male rats [33]. Increased adiponectin in response to HFD coupled with increased insulin-sensitivity and increased glucose metabolism of female adipocytes may contribute to the ability of female S5B rats to maintain normal metabolic parameters in the face of an energy-dense diet and increased adiposity. Due to the insulin sensitizing effects of adiponectin and its role as an adipokine, significantly lower levels of adiponectin in HFD fed rats, compared to LFD fed controls was used an indicator of MetSyn. Only S5B males consuming HFD met this criterion.

Total circulating cholesterol and serum triglyceride levels are used clinically to determine dyslipidemia and to indicate an elevated risk for CVD. We have previously reported that male OM rats have lower HDL cholesterol and higher triglyceride levels than male S5B rats [59], irrespective of diet. In the current study, total cholesterol levels were higher in male OM rats than females of the same strain. Previous studies indicate that females typically have a lower proatherogenic lipid profile than males, with lower total cholesterol and triglyceride levels [51,56,73]. Interestingly, in the current study, female S5B rats had higher total cholesterol levels than male S5B rats. Further studies are needed to further characterize these findings and determine whether higher total cholesterol levels in female S5B alter their risk for metabolic dysfunction. Total cholesterol levels greater than 175 mg/dL and triglyceride levels higher than 150 mg/dL were used as markers of MetSyn in this study. Levels of total cholesterol greater than 175 mg/dL were

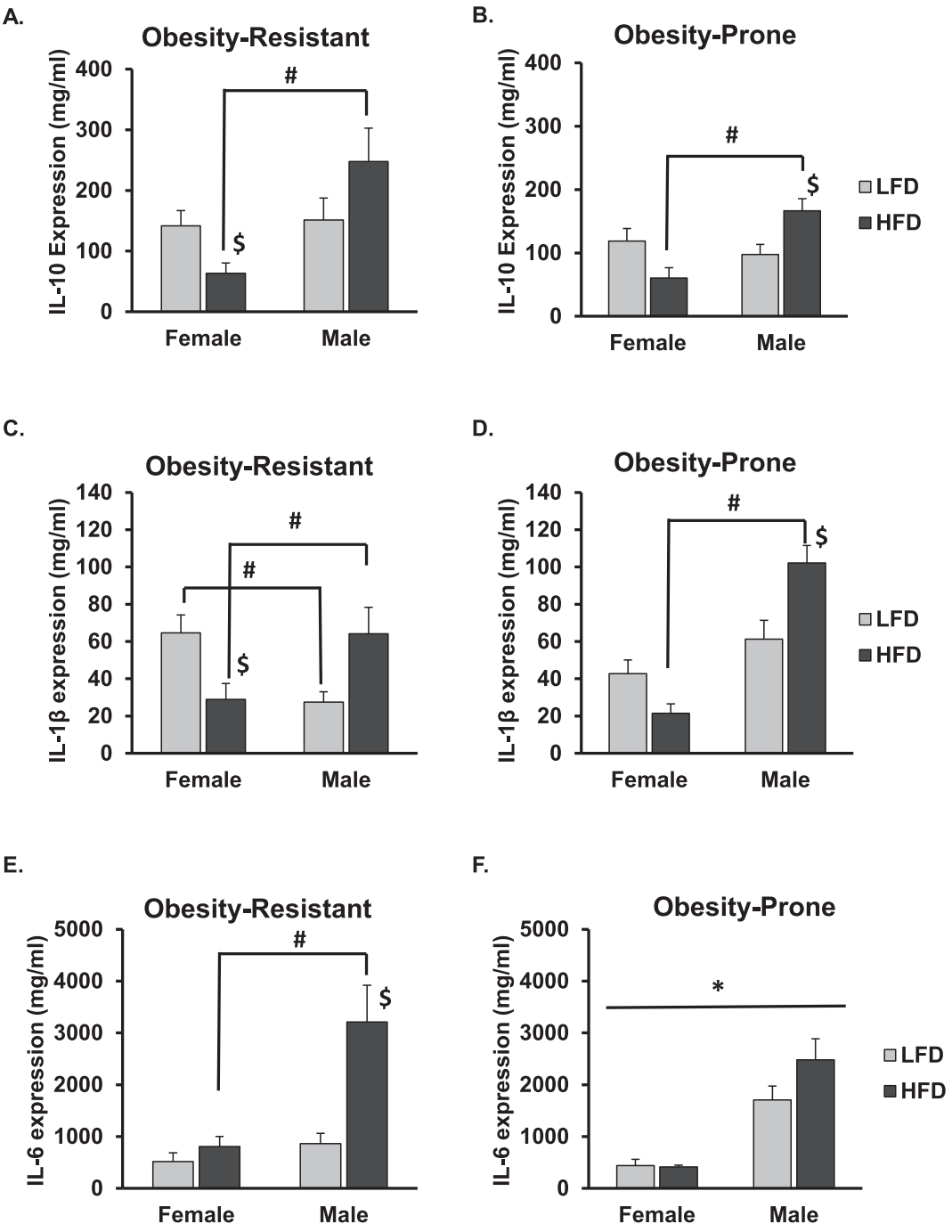

Fig. 4. Cytokine/chemokine expression was measured in gonadal fat depots in male and female S5B and OM rats. **A, B.** HFD intake reduced IL-10 expression in females and increased IL-10 expression in male S5B and OM rats. **C.** IL-1β levels were decreased in females and increased in male S5B rats following consumption of HFD. Female S5B rats expressed higher basal levels of IL-1β levels than male S5B rats. **D.** IL-1β expression was higher in male OM rats consuming HFD than female OM rats. **E.** Consumption of HFD increased IL-6 levels in male S5B rats. **F.** IL-6 levels were higher in male OM rats than female OM rats. * $p < .05$ main effect of sex; # $p < .05$ male vs. female, same diet; $ $p < .05$ LFD vs. HFD, same sex. Data shown as mean ± SEM.

only detected in female S5B rats. Elevated triglyceride levels were only detected in OM males consuming HFD (Table 1).

In the current study, three or more criteria associated with metabolic dysfunction, must be met for the classification of MetSyn (Table 1). Criteria for MetSyn was met in male obesity-resistant S5B (met 3 of 6 criteria) and male obesity-prone OM (met 4 of 6 criteria) rats consuming HFD. In both strains, males consuming LFD and females consuming either LFD or HFD failed to reach criteria for MetSyn. Female OM rats met criteria for higher body weight and greater visceral adiposity than their LFD fed controls, however, did not exhibit other criteria for classification of MetSyn. These findings support our hypothesis as does evidence from epidemiological studies indicating that the prevalence of many obesity-related complications is lower in females compared to males, despite higher levels of adiposity [31,46]. Therefore, female obesity-prone and obesity-resistant rats appear to be protected from the pathological effects typically seen with HFD consumption and increased visceral adiposity. Several plausible hypotheses have been proposed for the protection seen in females, including hormonal and adipose depot specific mechanisms. However, further investigation is necessary to fully elucidate the mechanisms that mediate this protection. Estrogen has

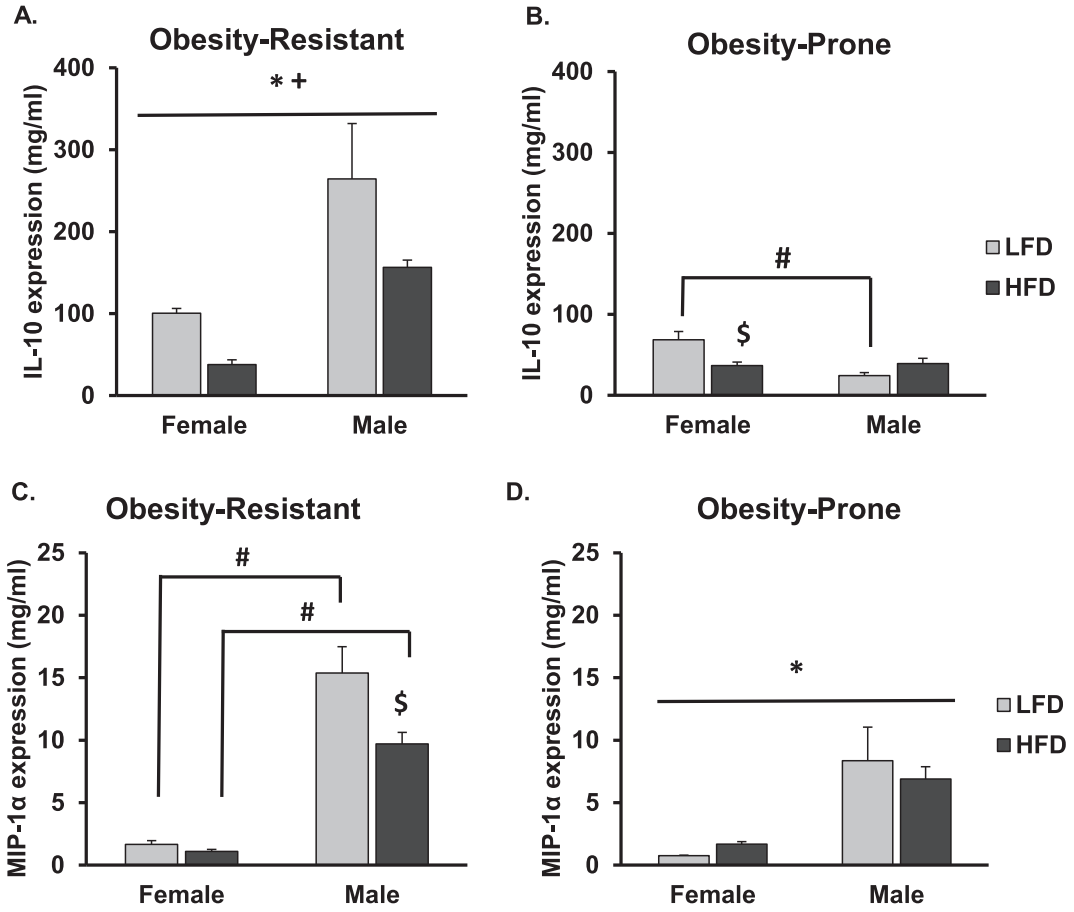

Fig. 5. Cytokine/chemokine expression was measured in mesenteric fat depots of male and female S5B and OM rats. **A** Expression of the anti-inflammatory cytokine, IL-10 was higher in male S5B rats and in rats consuming LFD. **B**. In OM rats, HFD intake reduced IL-10 levels in females. Male OM rats had lower basal levels of IL-10 than females. **C**. MIP-1α expression was lower in female S5B rats and was decreased following HFD consumption in males. **D**. In OM rats, males expressed high levels of MIP-1α than females. * $p < .05$ main effect of sex; # $p < .05$ male vs. female, same diet; +$p < .05$ main effect of diet. Data shown as mean ± SEM.

been shown to exert a protective role in females such that fat is preferentially deposited in SAT regions rather than in VAT regions and removal of estrogen is associated with increased metabolic dysfunction [19]. The current study investigated intact, young female rats of reproductive age, though future studies should include ovariectomized females to determine the role of estrogen on metabolic measures in these strains. Overall, female obesity-prone OM rats, particularly when fed HFD, may be representative of metabolically healthy obese individuals who exhibit excess body weight and adiposity, but have no metabolic comorbidities associated with excess adiposity.

Adipose inflammation, particularly inflammation in visceral adipose depots is associated with metabolic dysfunction [53]. Inflammation in visceral depots, including gonadal and mesenteric fat, has been linked to insulin resistance and metabolic syndrome and the proximity of these depots to internal organs and the portal circulation contribute to their effects [53]. Our previous study in male OM and S5B rats indicated that though there are inherent differences between the strains, consumption of HFD increased the expression of pro-inflammatory cytokines/chemokines in the epididymal fat depots of both strains [60]. In the current study, a cytokine/chemokine expression profile was assessed in gonadal, mesenteric and inguinal fat depots of male and female S5B and OM rats fed either LFD or HFD to determine sex differences in adipose inflammation. Overall, females expressed lower levels of cytokines/chemokines in gonadal fat depots, except for the pro-inflammatory cytokine, IL-1β, which was higher in female S5B rats consuming LFD. Expression levels of the anti-inflammatory cytokine IL-10, and the pro-inflammatory cytokines, IL-1β and IL-6 were higher in male S5B, compared to female S5B rats consuming the HFD. Gonadal fat expression of IL-10 and IL-1β was higher in male, compared to female, OM rats consuming HFD and IL-6 expression was overall higher in males than females (Fig. 4). In mesenteric fat depots, male S5B rats expressed higher levels of IL-10 and the macrophage inflammatory protein, MIP-1α, than female S5B rats. Expression of IL-10 and MIP-1α was decreased with HFD intake in S5B males and IL-10 was decreased in S5B females. IL-10 expression was higher in mesenteric fat in OM females, compared to males consuming the LFD. Consumption of HFD decreased IL-10 expression in OM females. MIP-1α expression was higher in male OM rats than females (Fig. 5). These data suggest a sexual dimorphism in visceral adipose inflammation and the response to HFD in males and females of both obesity-prone and obesity-resistant strains.

Subcutaneous depots, including inguinal fat, have been linked to insulin sensitivity and lie beneath the skin and not in close proximity with internal organs [53]. In this study, only IL-10 and Il-1β were expressed in inguinal depots at levels sufficient to measure in both males and females. Males expressed higher levels of the anti-inflammatory cytokine, IL-10, and the pro-inflammatory cytokine, IL-1β, than females. The consumption of HFD did not alter the expression of either of these cytokines in either strain or either sex (Fig. 6). The cytokine/chemokine assay used in the current study has been used successfully by our lab to measure adipose inflammation in male OM and S5B rats [60]. However, the expression of most of these inflammatory markers was undetectable in female rats, though interestingly, the anti-inflammatory cytokine, IL-10 was detectable in all depots assessed. IL-10 may play a compensatory role and be expressed in response to heightened adipose

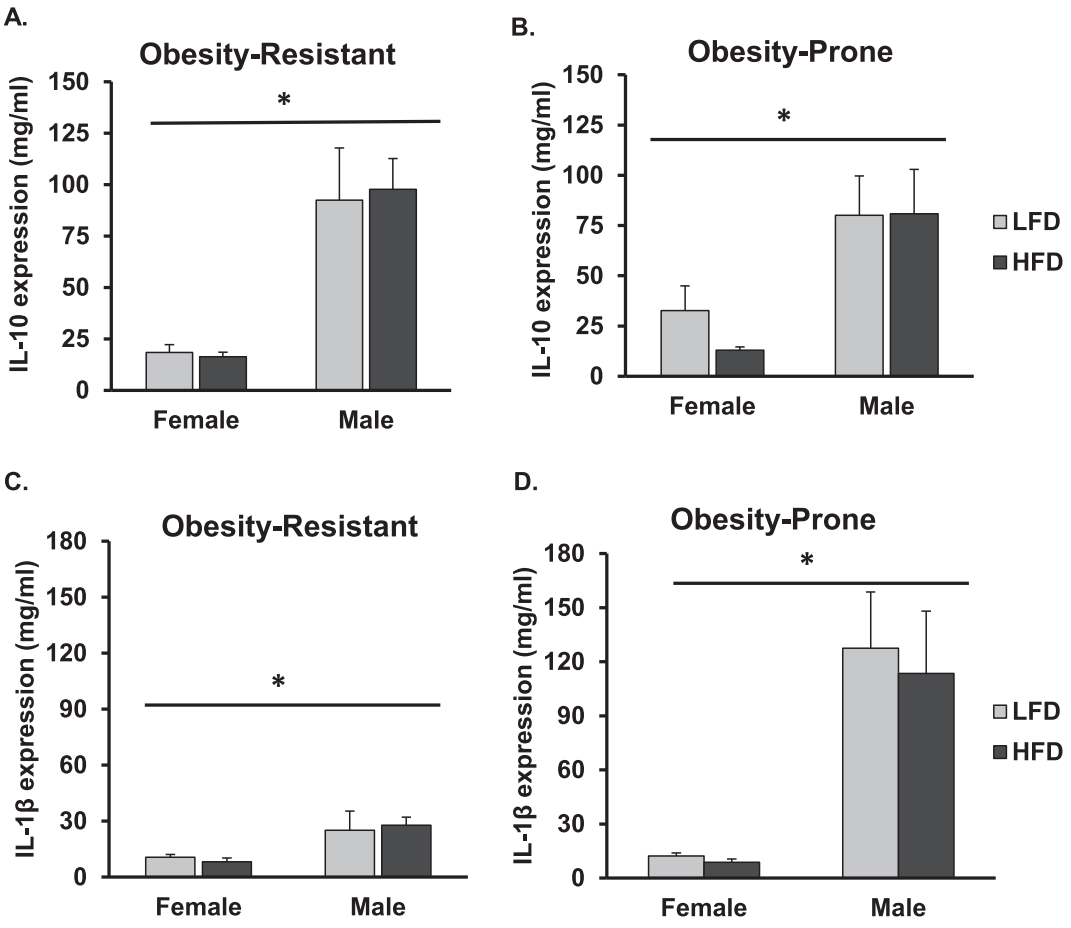

Fig. 6. Cytokine/chemokine expression was measured in the inguinal fat depot of male and female S5B and OM rats. A, B. IL-10 expression was higher in male S5B and OM rats than in females. C, D. The pro-inflammatory cytokine, IL-1β expression was higher in male S5B and OM rats, compared to female S5B and OM rats. * $p < .05$ main effect of sex. Data shown as mean ± SEM.

inflammation; however, recent studies suggest that IL-10 may have a detrimental effect on metabolic function [66]. Therefore, more studies are needed to fully elucidate the role of IL-10 on metabolic dysfunction in these strains. Overall, our data indicate a sexual dimorphism in adipose inflammation and suggest that a potential mechanism driving the increase in the incidence of obesity-related comorbidities in males is related to increased adipose inflammation, particularly in VAT.

5. Conclusions

The current study investigated sex differences in the development of obesity and risk factors for the development of obesity-related comorbidities in obesity-prone and obesity-resistant rats consuming HFD. Overall, male obesity-prone OM and obesity-resistant S5B rats consuming the HFD met criteria for MetSyn, supporting our previous studies suggesting that the consumption of HFD increases the risk for obesity-related comorbidities in male obesity-resistant rats [59,60]. We hypothesized that female rats would be protected against the development of MetSyn and obesity-related comorbidities and would be "metabolically healthy". Our data revealed that S5B and OM females did not meet criteria for MetSyn, even though OM females gained adiposity and weight when consuming the HFD. Sex differences in cytokine/chemokine expression were detected for visceral and subcutaneous fat depots. Lower levels of adipose inflammation in female OM and S5B rats coincided with a decreased risk of developing MetSyn and a probable mechanism for the decreased rates of comorbidities in females.

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