HHS Public Access

Author manuscript

Int J Obes (Lond). Author manuscript; available in PMC 2018 May 20.

Published in final edited form as:

Int J Obes (Lond). 2018 March ; 42(3): 535–541. doi:10.1038/ijo.2017.280.

High fat diet consumption differentially affects adipose tissue inflammation and adipocyte size in obesity-prone and obesity-resistant rats

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Abstract

Background/Objectives—Expanding visceral adiposity is associated with increased inflammation and increased risk for developing obesity-related comorbidities. The goal of this study was to examine high fat diet (HFD)-induced differences in adipocyte size and cytokine/chemokine expression in visceral and subcutaneous adipose depots in obesity-prone (OP) and obesity-resistant (OR) rats.

Methods—OP and OR rats were fed either a low fat diet (LFD, 10% kilocalories from fat) or HFD (60% kilocalories from fat) for 7 weeks. Adipocyte size and the presence of crown-like structures in epididymal and inguinal adipose tissue were determined. A multiplex cytokine/chemokine panel was used to assess the expression of inflammatory markers in epididymal and inguinal adipose tissues.

Results—A higher percentage of large adipocytes (> 5000 μm^2) was detected in the epididymal and inguinal adipose tissues of OP rats and a higher percentage of small adipocytes (< 4000 μm^2) was detected in the epididymal and inguinal adipose tissues of OR rats. More crown-like structures were identified in epididymal adipose tissue of OP rats fed a LFD, compared to OR rats. Consumption of a HFD increased the number of crown-like structures in OR, but not OP rats. Epididymal expression of pro-inflammatory cytokines (IL-1β, TNF-α) was higher in OP rats, compared to OR rats fed LFD. HFD consumption increased epididymal expression of GM-CSF, IL-1α, IL-1β, IL-6, MIP-2, and TNF-α in OP and OR rats. Inguinal expression of pro-inflammatory cytokines (IL-1α, IL-1β, TNF-α) was higher in OP rats, compared to OR rats.

Conclusions—Overall, these data suggest that a higher susceptibility to developing obesity is characterized by large adipocytes and increased visceral adipose inflammation. Interestingly, in

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The authors have no conflicts of interest to disclose.

OR rats, the detrimental effects of HFD consumption on visceral adipose inflammation are evident with only small increases in weight and adiposity, suggesting that HFD also increases the risk for obesity-related comorbidities in OR rats.

Keywords

Obesity-prone; obesity-resistant; visceral adipose; cytokines; adipocyte size; Inflammation

Introduction

Obesity is a multifactorial disease characterized by an excess of adiposity and an overconsumption of dietary fat[1–6]. A number of comorbidities are associated with obesity, including insulin resistance and cardiovascular disease, making obesity a serious health concern[4, 7]. Body composition and regional fat deposition, particularly visceral fat accumulation, are important mediators in the development of obesity and its related comorbidities[7–9]. Individual differences arise in regional fat deposition, and obese individuals with lower visceral fat content and higher insulin sensitivity are considered "healthy" obese[10–12]. In addition, the susceptibility to developing obesity differs among individuals, which may reflect an individual's propensity for developing obesity-related comorbidities[13–19]. Therefore, a heightened risk for developing obesity-related comorbidities may be seen in individuals with a greater susceptibility to develop obesity due to increased visceral adiposity.

Visceral fat and subcutaneous fat differ anatomically and in adipokine/cytokine expression and secretion[8, 20–22]. Visceral fat accumulation has been linked to the development of various obesity-related comorbidities (i.e. insulin resistance and cardiovascular disease)[23–25]. Compared to other fat depots, visceral fat has an increased infiltration of macrophages, increased secretion of pro-inflammatory adipokines/cytokines and acute phase proteins, and decreased secretion of anti-inflammatory adipokines/cytokines[8, 24, 26, 27]. These findings provided the basis for associating obesity with a chronic state of subclinical inflammation[8, 22, 27]. As part of the chronic inflammatory process that is seen with obesity, expansion of adipose tissue leads to adipocyte hypertrophy and hyperplasia. This hypertrophy increases the release of inflammatory cytokines and chemokines, which serve as a chemo-attractant for macrophages. These macrophages will form crown-like structures, which surround dead and decaying adipocytes, causing the adipocytes to release more inflammatory cytokines[28–30]. Obese individuals with crown-like structures present in their adipose tissue have higher circulating levels of triglycerides and insulin, and a higher percentage of these individuals have type 2 diabetes[31].

Obesity-prone Osborne-Mendel rats (OP) and obesity-resistant S5B/Pl rats (OR) have been used for many years to study physiological, behavioral, and neurochemical mechanisms that contribute to the individual susceptibility to develop obesity[13, 16, 17, 32–38]. When given a choice between a high fat (HFD) and a low fat diet (LFD), OP rats consume more calories from fat than carbohydrates, consume more HFD and subsequently, gain more body mass and fat mass in comparison to OR rats.[33, 39]. OP rats exhibit inherent and HFD-induced increases in visceral adiposity, body adiposity and skeletal muscle lipid accumulation,

compared to OR rats[13, 16, 17, 40, 41]. In skeletal muscle, OP rats consuming a HFD exhibited a decrease in pAMPK, an energy sensor and regulator of fat oxidation, and PPARγ, a regulator of fatty acid storage and glucose metabolism[13]. Previous reports have demonstrated that epididymal adipocyte diameters in OP rats are inherently larger than in OR rats[41]. Following HFD consumption adipocyte diameter increased in both strains, but to a greater extent in OP rats[41]. However, these studies did not investigate differences in the secretion of pro-inflammatory cytokines/chemokines in these strains.

A common mechanistic link between obesity and the progression to obesity-related comorbidities is chronic subclinical inflammation[42–44]. The goal of the current study was to examine the effects of HFD on adipocyte size and inflammation in strains that are prone to obesity, the OP rat, and resistant to developing obesity, the OR rat. We hypothesized that chronic consumption of HFD would increase viscera/epididymal adipocyte size in both strains, but exacerbate visceral/epididymal adipose inflammation, but not subcutaneous/inguinal adipose inflammation, in OP rats only. Rats were fed either a LFD or HFD for 7 weeks and weight gain, visceral adiposity, adipocyte histology, and markers of adipose inflammation were assessed.

Methods

Animals

Male obesity-prone, Osborne-Mendel (OP) and obesity-resistant, S5B/Pl (OR) rats (10–11 weeks old) used in these studies were bred in the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved Pennington Biomedical Research Center and LSU Health Sciences Center vivariums. Rats were individually housed on a 12/12 h light/dark (on at 0700) cycle with food and water available *ad libitum*. Animals were given access to a pelleted high fat diet (HFD, 60% kcal from fat, Research Diets, D12492, New Brunwick, NJ; n=6 rats/strain) or a pelleted low fat diet (LFD, 10% from fat, D12450B, Research Diets; n = 6 rats/strain) for 7 weeks. Rats were randomly assigned to diet condition. Body weight and estimated percent visceral adiposity ((epididymal weight (g) + retroperitoneal weight (g)/body weight (g)) × 100) was determined at sacrifice (beginning at 1300) All procedures were approved by the Pennington Biomedical Research Center and LSU Health Sciences Center Institutional Animal care and Use Committees.

Immunohistochemistry and immunostaining

Epididymal and inguinal adipose tissues were harvested and fixed in 4% paraformaldehyde, processed for paraffin imbedding and sectioned at a thickness of 5 μm. Hematoxylin and Eosin (H&E) staining and IBA1 immunohistochemistry were performed by the Pennington Biomedical Research Center Cell Biology and Bioimaging Core and based on standard protocols. Once stained, slides were scanned and images were processed using NanoZoomer Scanner and Viewer software (Hamamatsu, Bridgewater, NJ). H&E stained epididymal and inguinal adipose tissues were analyzed by determining the area (μm^2) of each adipocyte in the field (40×). Six fields were randomly selected for analysis from each tissue section from each rat. Adipocytes were categorized by size and the average number of adipocytes per size was determined. Data were expressed as the frequency of adipocytes in each size category

compared to the total number of adipocytes counted (% Total)[45]. Anti-rabbit IBA1 (#019-19741; Wako Chemicals, USA) staining was used to stain macrophages and to determine the number of crown-like structures for each sample (20×). For identification of crown-like structures, IBA1 staining had to completely surround the adipocyte. Histological sections were coded and the experimenters were blind to the experimental condition during analysis.

Cytokine/chemokine assessment

At the time of sacrifice, epididymal and inguinal adipose tissues were dissected and 100mg samples were collected, frozen in liquid nitrogen and stored at −80°C until further processing. Protein was isolated from adipose tissue samples as previously described[46]. Protein concentrations were measured using a micro BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL). Homogenates were used in a Rat Cytokine/Chemokine Magnetic Bead Panel-Immunology Multiplex Assay (Millipore Sigma, Billerica, MA) to measure the expression of 8 cytokine/chemokines (interleukin (IL)-1α, IL-1β, IL-10, IL-6, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein (MIP)-1α (also known as CCL3), and MIP-2 (also known as CXCL2). Cytokine/chemokine expression was normalized to protein values for each sample and expressed relative to OR-LFD levels.

Statistical Analyses

Data were analyzed with a 2 × 2 ANOVA with strain and diet as factors. Variance was assessed for each dependent variable measured. A Bonferroni post-hoc test was used to determine differences when a significant strain × diet interaction was detected. A significance level of $p<.05$ was used for all tests. Sample size was based on previous studies using these rat models.

Results

Animals

Body weight and percent visceral adiposity were measured following 7 weeks of HFD consumption. A significant strain × diet interaction was detected for final body weight ($F = 5.55$, $p<.03$; Figure 1A). OP fed either a LFD or HFD weighed more than OR rats. OP and OR rats consuming HFD weighed more than rats consuming LFD. OP rats had a higher percentage of visceral adiposity than OR rats ($F = 171.6$, $p<.001$) and rats consuming HFD had a higher percentage of visceral adiposity than rats consuming LFD ($F = 9.14$, $p<.01$; Figure 1B).

Immunohistochemistry and immunostaining

H&E staining was used to assess adipocyte size (area, μm^2) between OR and OP rats consuming a HFD or LFD. Adipocytes were categorized by size (<1000 μm^2, 1000–2000 μm^2, 2000–3000 μm^2, 3000–4000 μm^2, 4000–5000 μm^2, 5000–10 000 μm^2, > 10 000 μm^2) and data were expressed as the frequency of adipocytes in each category compared to the total number of adipocytes counted (% Total). Smaller adipocytes in epididymal adipose tissue were more prevalent in OR rats, compared to OP rats (1000–2000 μm^2 $F = 7.5$, $p<.05$;

2000–3000 μm^2 F = 36.02, p<.001; 3000–4000 μm^2 F = 33.06, p<.001; Figure 2). Larger adipocytes were more prevalent in OP, compared to OR rats (5000–10 000 μm^2 F = 30.69, p<.001; > 10 000 μm^2 F = 11.06, p<.01). In inguinal adipose tissue, smaller adipocytes were also more prevalent in OR, compared to OP rats (3000–4000 μm^2 F = 9.88, p<.05; 4000–5000 μm^2 F = 7.66, p<.05), while larger adipocytes were more prevalent in OP, compared to OR rats (> 10 000 μm^2 F = 9.45, p<.05; Figure 3). Consumption of a HFD did not alter epididymal or inguinal adipocyte size in either OP or OR rats. IBA1 immunostaining was used to determine the presence of crown-like structures, which signify dead or decaying adipocytes. In epididymal adipose tissue, a significant interaction between strain and diet was detected (F = 5.19, p<.05, Figure 4). More crown-like structures were detected in OP rats, compared to OR rats, suggesting strain differences, however, consumption of a HFD increased the number of crown-like structures in OR rats, but not OP rats. Very few crown-like structures were detected in inguinal fat in OR and OP rats and the number of crown-like structures was not affected by strain or diet (not shown).

Cytokine/chemokine assessment

Inherent strain differences and HFD-induced changes in cytokine and chemokine expression in epididymal and inguinal adipose tissue of OR and OP rats were assessed. In epididymal fat, consumption of HFD increased expression of IL-6 (F = 13.88, p<.01), GM-CSF (F = 15.59, p<.001), IL-1α (F = 6.51, p<.05), IL-1β (F = 13.04, p<.01), MIP-2 (F = 12.93, p<.01) and TNF-α (F = 16.58, p<.001) (Figure 5) in both OP and OR rats. Expression of the anti-inflammatory cytokine, IL-10, was higher in epididymal fat in OR, compared to OP rats (F = 5.56, p<.05), and expression of the pro-inflammatory cytokines, IL-1β (F = 11.12, p<.01) and TNF-α (F = 15.98, p<.001) were higher in OP, compared to OR rats. A significant interaction between strain and diet was detected in epididymal fat for MIP-1α (F = 5.04, p<.05). Post-hoc tests indicate that OR rats inherently expressed higher levels of MIP-1α than OP rats and that HFD consumption increased MIP-1α expression in OR rats, but not OP rats. In inguinal fat, OP rats expressed higher levels of pro-inflammatory cytokines, IL-1β (F = 14.19, p<.001), TNF-α (F = 5.83, p<.05) and IL-1α (F = 18.67, p<.001), compared to OR rats irrespective of diet. A significant interaction between strain and diet was detected for MIP-1α expression (F = 4.81, p<05) (Figure 6). OP rats had higher expression of MIP-1α in inguinal fat than OR rats when fed the LFD. No diet effects were detected in inguinal adipose tissue.

Discussion

Chronic subclinical inflammation is a mechanistic link between obesity and the progression to obesity-related comorbidities[42–44]. The current study examined strain and HFD-induced differences in visceral and subcutaneous fat inflammation and adipocyte size in two rat models that differ in their susceptibility to develop obesity. OP rats are prone to developing obesity, consume more HFD, gain more weight when consuming a HFD, and have more visceral adiposity and total body adiposity than OR rats[13, 16, 17, 33, 39–41]. We hypothesized that OP rats would exhibit increased visceral adipose tissue inflammation and adipocyte hypertrophy compared to OR rats and that these effects would be exacerbated by chronic consumption of HFD. Increased visceral adipose tissue inflammation in OP rats may

increase their risk for developing obesity-related comorbidities, like insulin resistance and cardiovascular disease.

An increase in mast cells, eosinophils, neutrophils and macrophage recruitment to adipose tissue initiates an inflammatory cascade providing a potential link between obesity, insulin resistance and cardiovascular disease. The proportion of adiposity in an individual is related to the accumulation of adipose tissue macrophages. As obesity develops, adipocytes undergo hypertrophy due to increased triglyceride storage and an increased percentage of adiposity can lead to a metabolically dysfunctional phenotype[28–30]. In the current study, and as previously shown[13, 16, 17, 40, 41], OP rats weighed more than OR rats, gained more weight when consuming a HFD, and had a higher percentage of visceral adiposity, as measured by the epididymal and retroperitoneal fat depots. Though food consumption was not specifically measured in the current study, numerous studies from our lab have demonstrated significant hyperphagia of HFD in the OP rats[13, 16, 47]. Histological analyses of adipocyte size and number in epididymal adipose tissue demonstrated that the distribution of small and large adipocytes differed in OP and OR rats. OP rats have larger epididymal adipocytes than OR rats. The majority of adipocytes in OP rats were larger than 5000 μm^2, while the majority of adipocytes in OR rats were smaller than 4000 μm^2. These data suggest that there are strain differences in visceral adipocyte size in OP and OR rats, which may indicate that more inflammation is present in the visceral adipose tissue of OP rats. The consumption of HFD did not lead to epididymal adipocyte hypertrophy in OP or OR rats. These data led us to propose that the visceral adipocytes in OR rats are resistant to hypertrophy and that the visceral adipocytes in OP rats may have reached the maximal size. Similar findings were noted for the inguinal adipocytes, in which OP rats had a greater number of large adipocytes and OR rats had a greater number of small adipocytes. As with the epididymal adipocytes, HFD consumption did not lead to significant changes in inguinal adipocyte size in either strain.

As part of the inflammatory process, locally secreted chemokines attract macrophages, which lead to the formation of crown-like structures. These crown-like structures surround dead or dying adipocytes. These macrophages will release cytokines that perpetuate the inflammatory process and can lead to insulin resistance[28, 29]. In the current study, epididymal and inguinal adipose tissues were stained for the presence of macrophages and the number of crown-like structures was assessed. In epididymal adipose tissue of LFD fed rats, more crown-like structures were detected in OP rats, compared to OR rats, however, the consumption of the HFD significantly increased the presence of crown-like structures in OR rats, but not OP rats. Taken together, these data suggest that even though HFD intake did not lead to hypertrophy of the epididymal adipocytes in the OR rats, HFD consumption did increase inflammation and adipocyte dying/death in this strain. Consumption of HFD in OP rats did not exacerbate adipocyte dying/death, as assessed by the crown-like structures. Very few crown-like structures were detected in inguinal adipose tissue and these were not affected by strain or diet.

To further investigate adipose tissue inflammation in OP and OR rats, a cytokine/chemokine assay was performed. In epididymal adipose tissue, OP rats expressed lower levels of the anti-inflammatory cytokine, IL-10, and higher levels of the pro-inflammatory cytokines,

IL-1β and TNF-α, compared to OR rats. Higher expression of IL-10 in OR rats may reflect a protective and/or compensatory mechanism by the macrophage population in the visceral adipocytes of OR rats. Additionally, the production and secretion of anti-inflammatory cytokines are more typical of M2 macrophages, while the production and secretion of pro-inflammatory cytokines are more typical of M1 macrophages[48]. The current study did not investigate the differential populations of macrophages, however, there may have been inherent and/or diet-induced shifts in macrophage populations, which may account for differences in IL-10 expression. The consumption of the HFD augmented epididymal cytokine/chemokine expression (GM-CSF, IL-1α, IL-1β, IL-6, MIP-2, TNF-α) in OP and OR rats. HFD intake produced the largest increase in TNF-α expression, which has previously been shown to be increased in the adipose tissue of obese animals and is associated with insulin resistance[28]. A significant interaction between strain and HFD intake on MIP-1α expression in epididymal fat was detected. MIP-1α has previously been shown to be almost entirely derived from macrophages rather than adipocytes[49]. Therefore, a HFD-induced increase in MIP-1α in OR rats may be the result of the increased prevalence of crown-like structures seen in the epididymal fat of those rats. In OP rats, the number of crown-like structures, though inherently higher in OP than OR rats, did not increase with the consumption of a HFD. Epididymal expression of MIP-1α in OP rats is thereby reflective of the presence of crown-like structures. In inguinal adipose tissue, expression of the pro-inflammatory cytokines, IL-1α, IL-1β and TNF-α, were higher in OP, compared to OR rats. HFD consumption did not exacerbate cytokine expression in the inguinal adipose tissue of either strain, which was consistent with our immunohistological data, showing that crown-like structures in this fat depot were not altered. A significant interaction between strain and diet on MIP-1α was detected in inguinal fat. Specifically, higher levels of MIP-1α were measured in OP rats, compared to OR rats. As mentioned, MIP1-α is released by macrophages[49] and though there were a low number of crown-like structures counted in the inguinal fat depots of OR and OP rats, OP rats did have more crown-like structures. This increase in crown-like structures, though not statistically significant, may be physiologically significant and may account for the higher levels of MIP1-α in the inguinal fat depots of OP rats. Overall, our data support previous findings detailing strain differences in visceral and subcutaneous adipocyte size[41] and support our hypothesis that OP rats have inherently higher visceral adipose tissue inflammation than OR rats. In visceral adipose tissue, IL-1β and TNF-α were affected by both strain and diet, while IL-10 was only affected by strain and GM-CSF, IL-1α, IL-6 and MIP-2 were only affected by HFD consumption. Interestingly, HFD consumption increased inflammation in the visceral adipose tissue of OR rats, suggesting that dietary fat intake may be able to override the predisposition for resistance to obesity, as measured by body weight and percent adiposity.

Measures of visceral adiposity are indicators of an increased risk for obesity-related comorbidities, including metabolic dysfunction, insulin resistance and cardiovascular disease. As a consequence of expanding visceral adiposity associated with obesity, alterations in glucose tolerance and systemic inflammation are prevalent[10]. The current study investigated strain and HFD-induced alterations in adipocyte hypertrophy and adipose tissue inflammation. The OP rats have inherently higher levels of visceral fat, larger adipocytes and more crown-like structures in visceral fat, compared to OR rats. Though

adipocyte size and crown-like structures were affected by strain, these measures were not affected by HFD intake in OP rats. The expression of the pro-inflammatory cytokines, IL-1β and TNF-α, were higher in the visceral adipose tissue of OP rats, compared to OR rats. Furthermore, the expression of multiple pro-inflammatory cytokine/chemokines in the visceral adipose tissue of OP rats was increased by the consumption of HFD. These data support the hypothesis that OP rats are inherently at a higher risk for developing obesity-related comorbidities, and that these risks are exacerbated by consuming a HFD. In OR rats, consumption of a HFD did not lead to an increase in the percentage of visceral adiposity or adipocyte hypertrophy, however, HFD intake did increase the number of crown-like structures and pro-inflammatory cytokine expression. These data suggest that even though an individual that consumes a HFD appears to be resistant to developing obesity, as measured by body weight and percent fat, the detrimental effects of HFD consumption on visceral adipose inflammation are apparent and may lead to an increased risk of developing obesity-related comorbidities. Future studies are needed to examine differences in the susceptibility to develop obesity on the progression toward the development of obesity-related comorbidities.

Acknowledgments

This research was supported by a COBRE pilot & feasibility grant to SDP (1P30 GM106392). This work utilized the facilities of the Cell Biology and Bioimaging Core that are supported in part by COBRE (NIH8 1P30GM118430-01) and NORC (P30DK072476) center grants from the National Institutes of Health.

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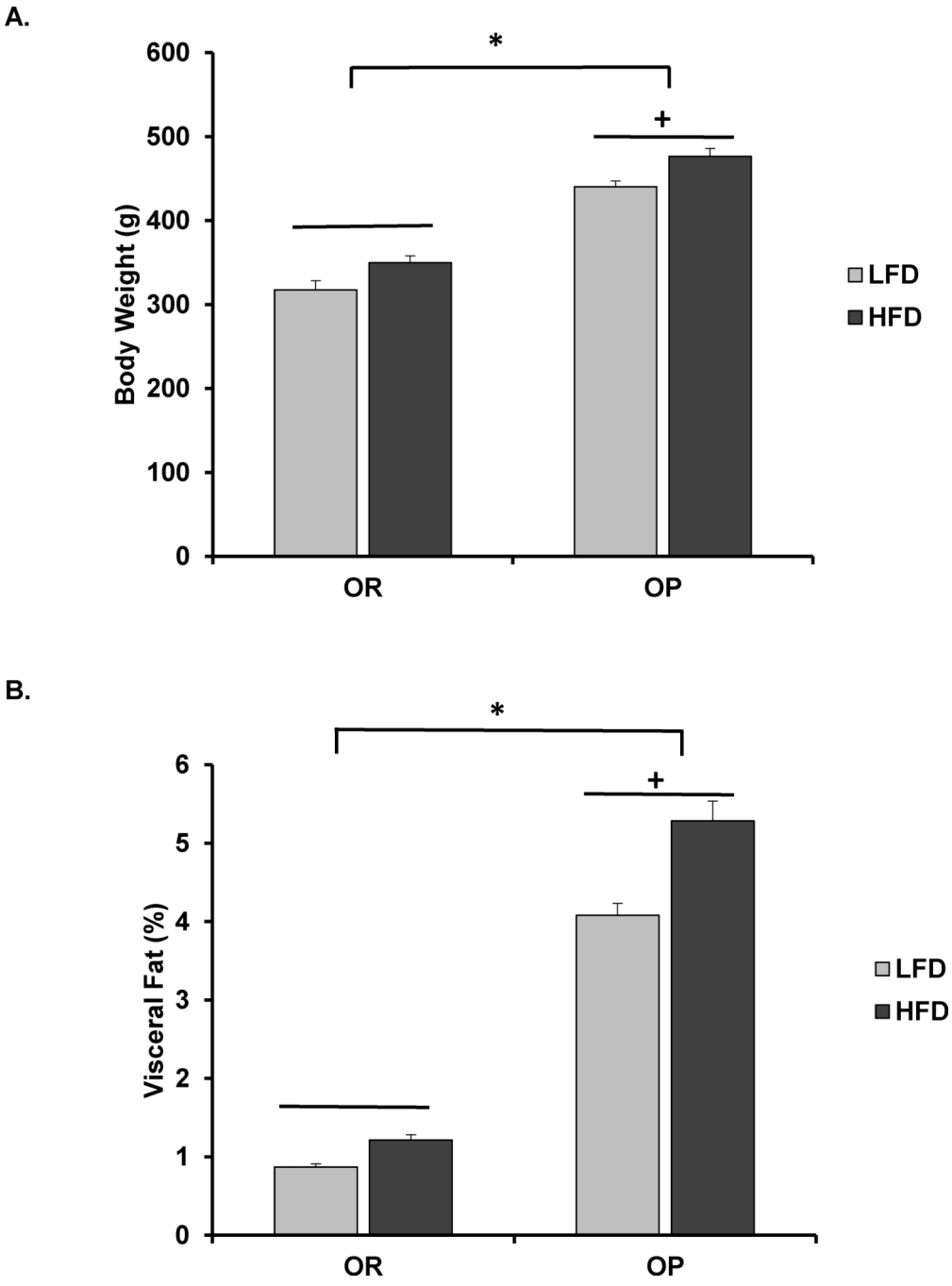

Figure 1.

OP and OR rats were fed either a HFD or a LFD for 7 weeks. **A.** At the end of the study, OP rats weighed more than OR rats and rats consuming the HFD weighed more than rats consuming the LFD. **B.** Visceral adiposity was determined by measuring epididymal and retroperitoneal fat depots. OP rats had a higher percentage of visceral adiposity than OR rats and rats consuming HFD had higher levels of visceral adiposity than rats consuming LFD. + $p < .05$, across strains; * $p < .05$, across diets. Data shown as mean ± SEM.

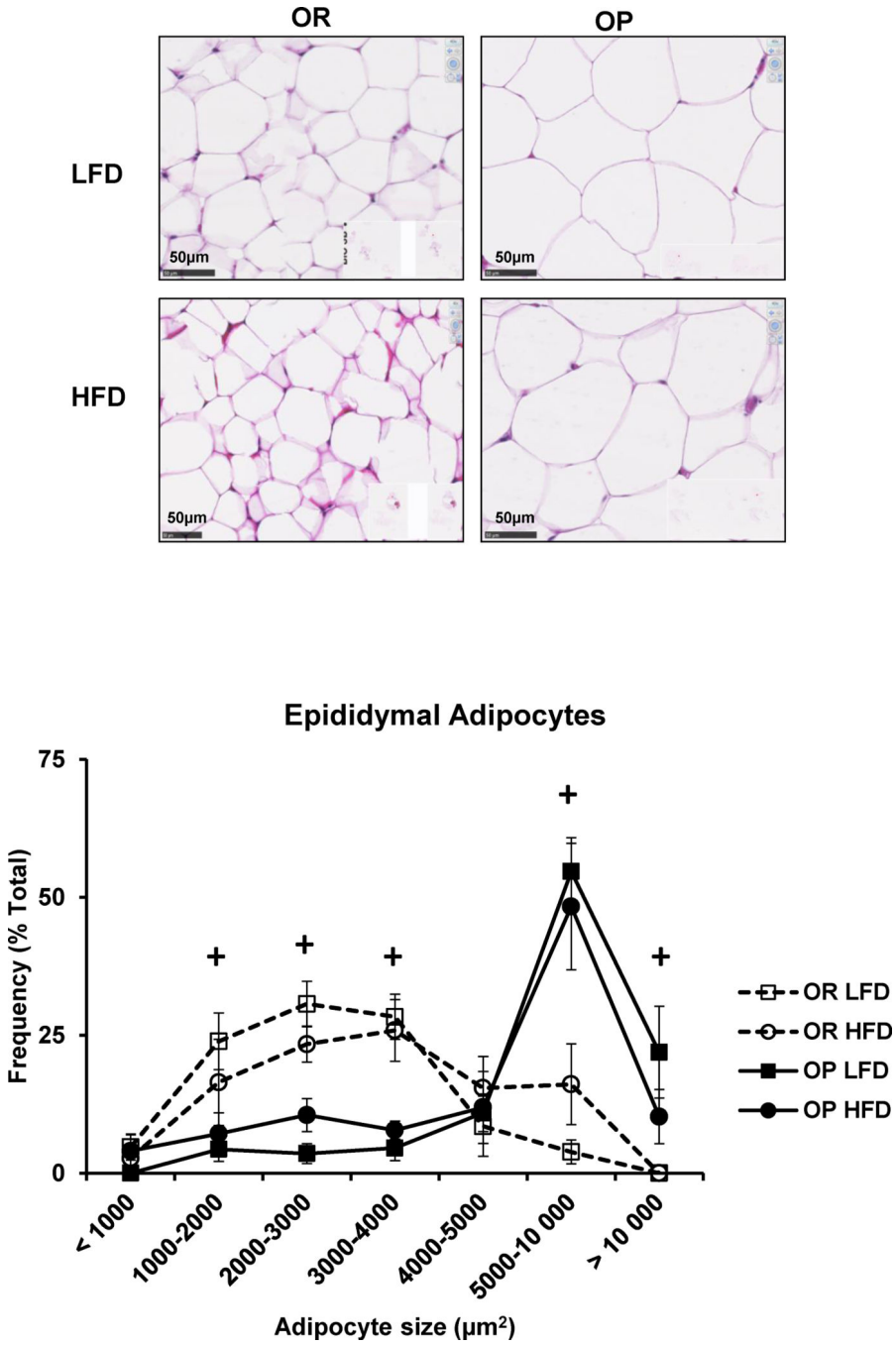

Figure 2.

Adipocytes from epididymal fat depots were stained with H&E for the determination of adipocyte size. Epididymal fat depots in OR rats contained a higher number of small adipocytes and in OP rats fed a LFD, a higher number of large adipocytes. Consumption of a HFD did not alter adipocyte size in epididymal adipose tissue. + $p<.05$, across strains. Data shown as mean ± SEM.

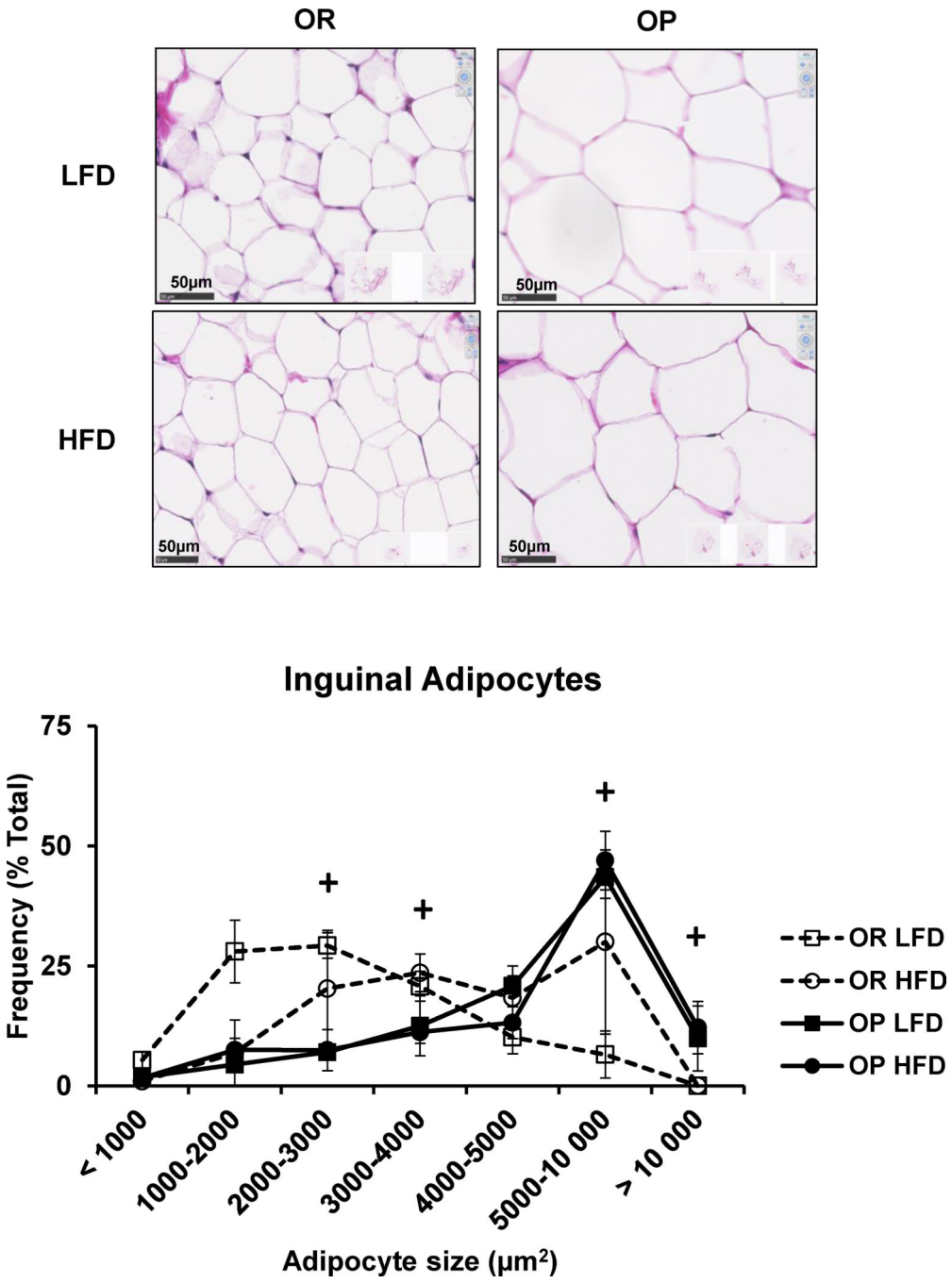

Figure 3.

Adipocytes from inguinal fat depots were stained with H&E for the determination of adipocyte size. A higher number of small adipocytes were identified in OR rats, while a higher number of large adipocytes were detected in OP rats fed a LFD. Consumption of a HFD did not alter adipocyte size in epididymal adipose tissue. + $p<.05$, across strains. Data shown as mean ± SEM.

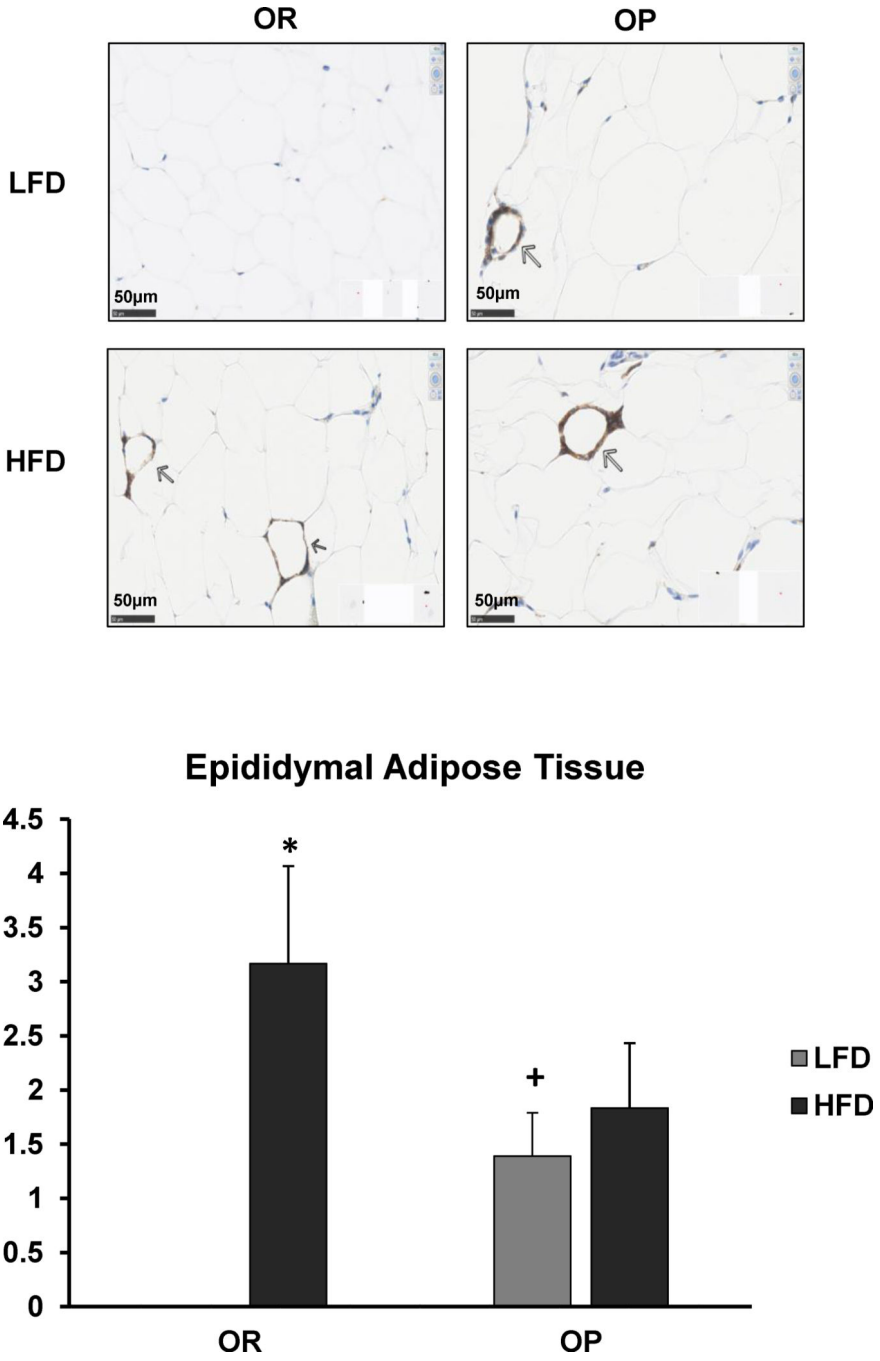

Figure 4.

Crown-like structures were assessed by IBA1 immunostaining in epididymal and inguinal fat depots of OP and OR rats. More crown-like structures were detected in the epididymal adipose tissue of OP rats fed a LFD, compared to OR rats. HFD intake selectively increased the number of crown-like structures in OR rats, not OP rats. + $p < .05$, across strains; * $p<.05$, across diets. Data shown as mean ± SEM.

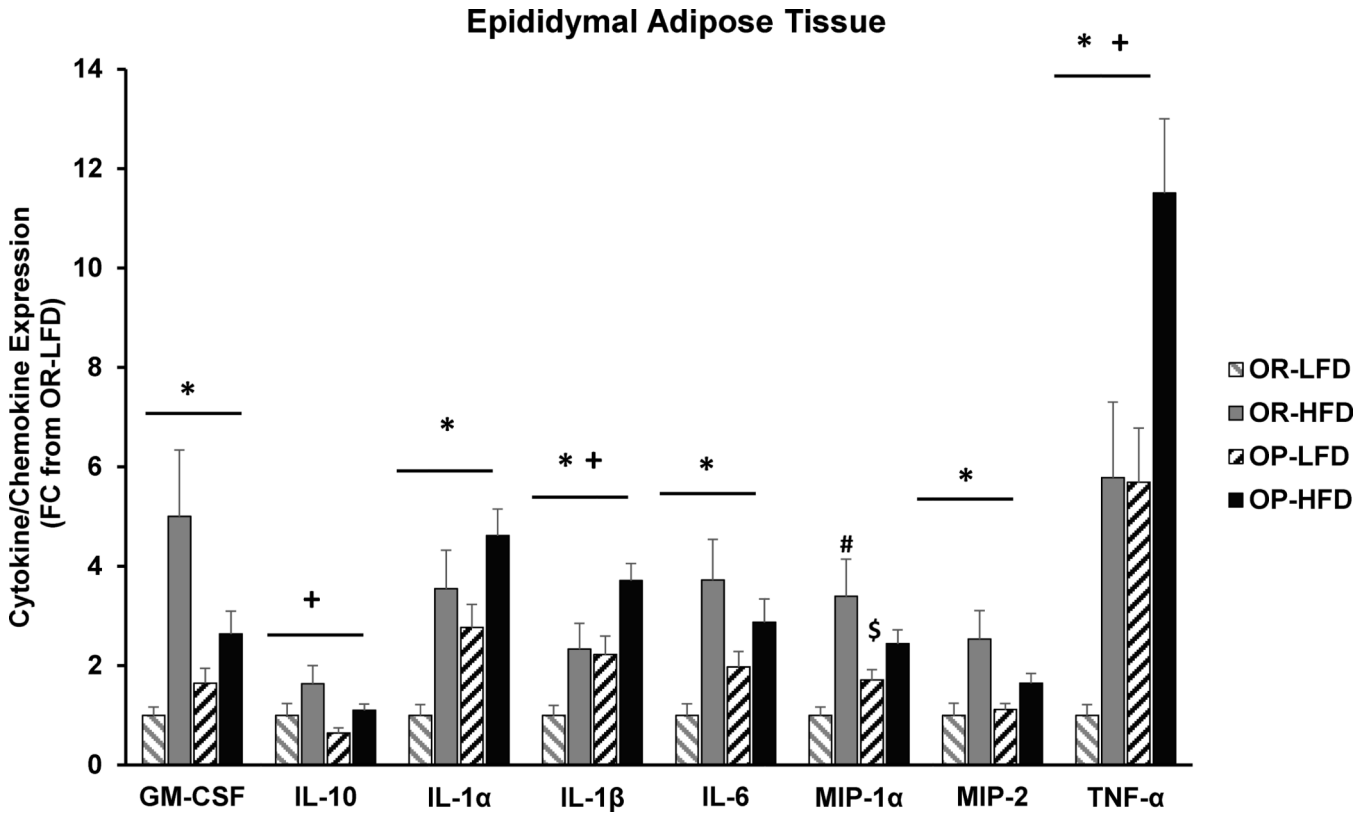

Figure 5.

A multiplex cytokine/chemokine assay was used to determine expression levels of anti- and pro-inflammatory cytokines and chemokines in the epididymal adipose tissue of OP and OR rats. Expression of multiple pro-inflammatory cytokines was increased in OP rats, compared to OR rats fed a LFD. The consumption of a HFD increased expression of multiple pro-inflammatory and chemokines in both OP and OR rats. + $p < .05$, across strains; * $p<.05$, across diets. # $p<.05$ OR-LFD vs. OR-HFD; $ OR-LFD vs. OP-LFD. Data shown as mean ± SEM.

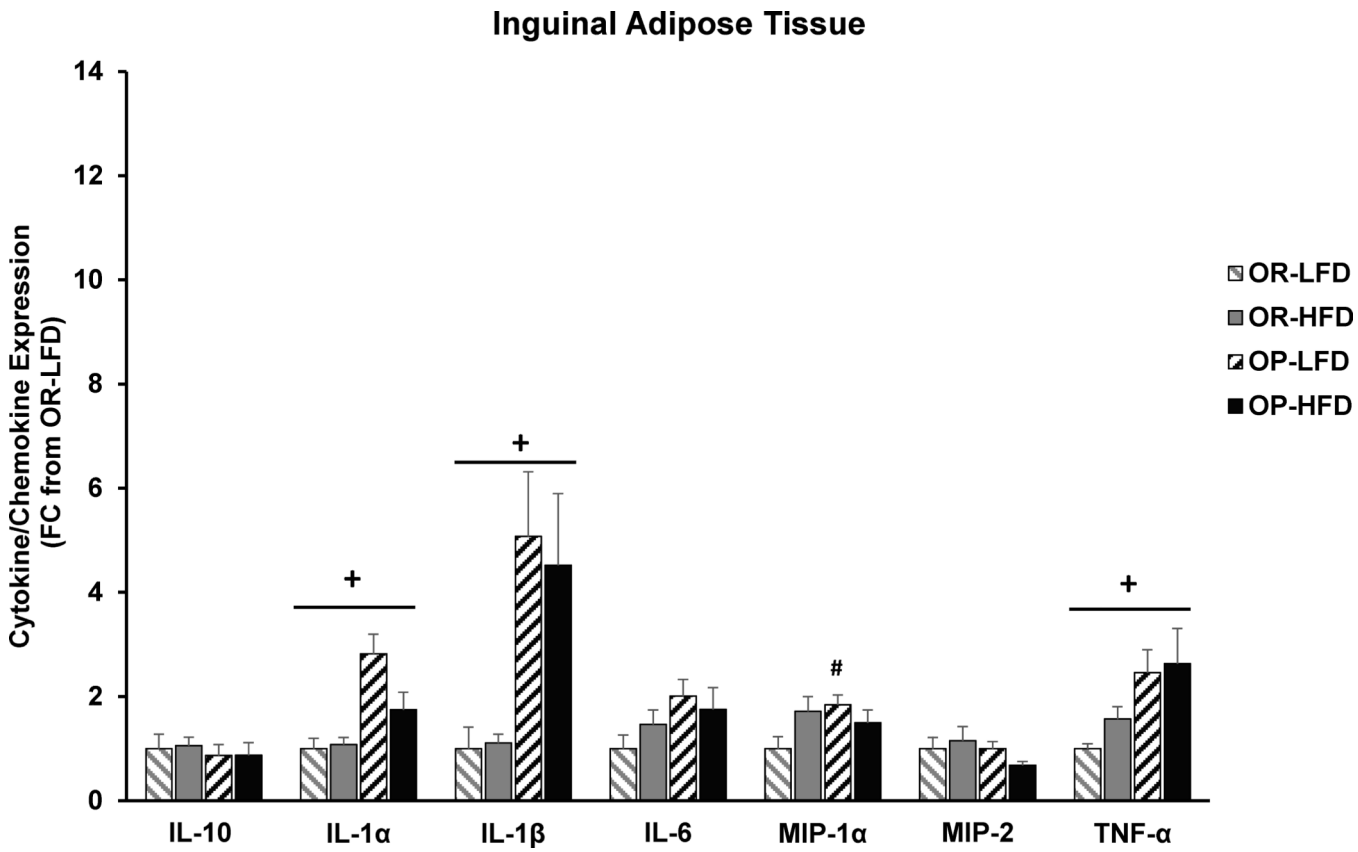

Figure 6.

A multiplex cytokine/chemokine assay was used to determine expression levels of anti- and pro-inflammatory cytokines and chemokines in the inguinal adipose tissue of OP and OR rats. Higher expression levels of IL-1α, IL-1β and TNF-α were detected in OP rats, compared to OR rats fed a LFD. + $p<.05$, across strains; # $p<.05$ OR-LFD vs. OP-LFD. Data shown as mean ± SEM.