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SEX DIFFERENCES IN THE INFLUENCE OF OBESITY ON A MURINE MODEL OF ALLERGIC LUNG INFLAMMATION

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Abstract

**Background:** Despite the overwhelming evidence showing the influence of sex or obesity in the development of respiratory diseases in humans and animals, the mechanisms by which these combined two factors influence allergic asthma are not well understood. **Objective:** We have investigated the interaction between sex and weight gain in an experimental model of lung allergic inflammation induced by chicken egg ovalbumin (OVA) in mice. **Methods:** Animals were fed a high fat diet for 8 weeks and then sensitized and challenged with OVA. **Results:** Our results demonstrate that in comparison to males, HFD allergic female mice exhibit a reduction in the number of leukocytes in the lung lumen when challenged with OVA and, in contrast, an accumulation of these cells in the lung tissue. In addition, we also observed that allergic HFD female mice presented a robust lung remodelling in comparison to HFD males, evidenced by higher deposition of collagen in the airways and TGF-β in lung fluid. Measuring epithelial adhesion molecule expression, we observed that female mice presented a significantly lower expression of CD103 than males in BAL cells, regardless of the diet. Similarly, HFD female mice express lower levels of EpCam in lung tissue in comparison to males and lean females. Levels of A20/TNFAIP3 expression in lung tissue demonstrated that HFD female mice express lower levels of these regulatory factors than all the other groups. However, this reduction was not accompanied by an increase in activated NF-κB. **Conclusions:** Our results present evidence that the interaction between sex and weight gain alters the progression of allergic asthma in mice with females developing airways remodelling at a much earlier stage than males. **Clinical Relevance:** These data may contribute to a better understanding of the clinical differences in the development and severity of allergic asthma observed between men and women of reproductive age.
1. Introduction

Sex differences in asthma have been well described in the literature. Several reports have shown that asthma is more prevalent in boys than in girls but this status is inverted during puberty, indicating an important role for sex hormones in the development of this disease\(^1\). Similarly, the influence of obesity has also been documented with studies indicating that body weight is an important risk factor in asthma\(^2\). However, despite the overwhelming evidence showing the influence of sex or obesity in the development of respiratory diseases in humans, the mechanisms by which these two factors interact to influence asthma is not well understood. To date, the vast majority of publications in this field are longitudinal or cross-sectional studies of human populations\(^3\)–\(^6\). Some reports have shown that nearly 75 percent of emergency room visits for asthma have been among obese individuals\(^7\) and several cross-sectional studies have found an increase in the prevalence of asthma among obese patients\(^4\). More importantly, obesity appears to interfere with the efficacy of standard therapies of asthma\(^8\), raising concerns about whether treatment of these patients is adequate. In addition, it has become increasingly clear that obesity as an important risk factor in the onset and worsening of asthma seems to be particularly higher among women\(^3\). Several studies point towards an association between weight gain and asthma in females, but not in males\(^9\)–\(^11\), although some of the largest studies have not confirmed this association\(^12\). It is plausible that an increased association in women could be due to female sex hormones\(^13\),\(^14\) or that the effects of adiposity are greater in women simply because they have a higher proportion of body fat than men, but the reasons for the apparent sex difference observed in many studies is unclear\(^15\).
Sex differences in the development of allergic inflammation in the lung and obesity have also been demonstrated in murine models. Experimental studies have shown that female allergic mice develop a more robust eosinophilic inflammatory response to ovalbumin and also a higher amount of airway remodelling compared with male mice or ovariectomized females. Mice present sex differences in both the acquisition of weight gain and distribution of excess fat stores due to genetic factors. Sex differences in obesity, adiposity and insulin resistance have also been observed in animal models of metabolic syndrome, suggesting that the development of obesity is influenced by sex in mice as it is in humans.

In this study, we sought to investigate the interaction between weight gain and sex in an experimental model of allergic inflammation in the lung induced by chicken egg ovalbumin (OVA) in mice. More specifically, we have investigated the influence of sex and weight on leukocyte migration, adhesion molecule expression and airway remodelling in standard chow and high fat diet fed male and female mice.
2 Methods

Additional information of the methodology used in these experiments can be found in the supplemental section.

2.1 Animals and induction of experimental obesity

4-week-old male and female mice (C57BL/6) (Envigo, UK) were acclimatized for 2 weeks with conventional diet and water *ad libitum* before the onset of the hyperlipidic diet. HFD group animals were fed for 8 weeks with a hyperlipidic diet (HFD – High Fat Diet) (SDS diet, Cat. Numb. 824054) composed of 60% fat, 20% protein and 20% of carbohydrates. SC group animals were fed with conventional diet for the same period (SC – Standard Chow) (Picolab Rodent Diet 20, Cat: 50503) composed of 5% fat, 20% protein, 5% fibre, 6% ash, 60% Nitrogen free extract and 10% moisture. The effectiveness of the diet was measured weekly by weight measurement and after 8 weeks by body weight gain, serum triglycerides, glucose and total cholesterol levels. At the end of the experiment we also measured perigonadal fat weight and serum levels of leptin. Experiments were approved by the Home Office under The Animals (Scientific Procedures) Act (1986) and by the Ethics Committee of King's College London.

2.2 Experimental design

Eight groups were evaluated:

1. OVA-sensitized and OVA-challenged standard chow male (Male SC OVA)

2. OVA-sensitized and OVA-challenged high fat diet male (Male HFD OVA)

3. OVA-sensitized and OVA-challenged standard chow female (Female SC OVA)
4. OVA-sensitized and OVA-challenged standard chow fed female (Female HFD OVA)

5. Non-sensitized and OVA-challenged standard chow fed male (Male SC Sham)

6. Non-sensitized and OVA-challenged high fat diet fed male (Male HFD Sham)

7. Non-sensitized and OVA-challenged standard chow fed female (Female SC Sham)

8. Non-sensitized and OVA-challenged high fat diet female (Female HFD Sham)

2.3 Sensitization and challenge to chicken egg albumin (OVA)

At week 6 of the diet, female and male mice were immunized intraperitoneally 3 times, with a 5 day interval between injections, with 30 µg of OVA (type V; Sigma Chemical Co., Gillingham, UK) adsorbed to a saturated solution of aluminium hydroxide (Sanofi, Brazil). Control animals (Sham groups) received vehicle only. On days 14, 15 and 16 after the first immunization, all animals were challenged 3 times, once daily with a 3% solution of OVA for 20 minutes.

2.4 Bronchoalveolar alveolar lavage (BAL)

Twenty-four hours after the last OVA challenge, mice were anaesthetized with urethane (2 g/kg i.p.; Sigma Gillingham, UK), a cannula inserted into the exposed trachea and three 0.5 mL aliquots of sterile saline were injected into the lungs. The total number of cells in the lavage fluid was counted with an improved Neubauer haemocytometer. For differential cell counts, cytospin preparations were stained with Diff Quick (DADE Behring, Germany) and cells were counted using standard morphological criteria. In order
to minimise the impact of sex differences in size on the interpretation of our data, we opted to calculate all cell counts per gram of body weight in order to have a better comparison of the response between the groups.

2.5 Analysis of lung homogenates

Fragments of the lungs were removed and cut into small pieces. The fragments were placed in a falcon tube with 10 mL of RPMI 1640 medium enriched with enzymes Liberase (2μg/mL, Roche, Germany) and DNase I Type IV (25μl/mL, Sigma, UK); the mixture was then homogenized at 37 °C until complete dissociation. Red cells were lysed and any remaining cells enumerated manually as described above. In order to minimise differences due to samples size we divided the cell count by grams of tissue collected.

2.6 Lung histology

Pulmonary tissue integrity and inflammation levels were measured by histology using Haematoxylin/Eosin (Sigma-Aldrich, UK). The lungs were fixed in 10% formalin, and then cut into 5μm slices and stained. The specific visualization of eosinophils was made using the Luna staining specific for eosinophils, as described elsewhere (Fisher Sceintific, UK)\(^22\). With this method eosinophils stained strongly in red and were imaged using a light microscope.

2.7 Cytokines, IgE and hormone determination

TGF-β1 (Mouse TGF- β1 DuoSet ELISA – R&D Cat DY1679-05), leptin (Mouse Leptin ELISA – Millipore Cod EZML-82K), tryglicerides GOD/PAP (LaborLab
1770290), glucose GOD/PAP (LaborLab 1770130), total cholesterol GOD/PAP (LaborLab 1770080) were measured in BAL and serum, respectively following instructions of the manufacturers.

2.8 Collagen deposition

Collagen deposition in lung tissue was determined by histological staining using the Masson's Trichrome method (Sigma-Aldrich, UK). The presence of collagen fibres, stained in blue, was determined by optical microscopy and their density was assessed by ImagePro (v.2000) software.

2.9 Immunohistochemistry

Histological sections were incubated with anti-TNFAIP3 (InsightTech bs-2803R-B) and anti-NF-κB p65 phospho S536 (Abcam Cat: ab86299). After washing, the tissue was incubated with the secondary antibody (Sigma, UK, Cat: SAB4600006) for 1 hour at 37 °C. For quantification, 5 areas of interest per animal were photographed using a 20x objective and % of area of positive staining was quantified using Image-Pro Plus software, v5.5.0.29.

2.10 Immunofluorescence (IF)

BAL cells were attached to a microscope slide by cytospin, fixed with cold acetone and stored at -80 ºC. Cells were incubated with anti-CD103(Clone 2E7 CAT 121407 Biolegend). Frozen lung tissue slices were incubated with anti- EpCam (Clone G8.8 CAT 118201 Biolegend). Secondary antibody was added (Alexa-Fluor 594, CAT A11007, Life Technologies, UK). All areas emitting fluorescence (regardless of intensity)
were analysed and photographed using a 40x objective. The result is the product of both fluorescence intensity and fluorescent area, according to the following equation (CTCF = arbitrary units: CTCF = Leukocyte integrated density - (leukocyte area x background value). Fluorescence was quantified using Image J, v 1.45s (NIH, USA).

2.11 Statistical analysis

Statistical analysis of data was performed by GraphPad Prism version 7. The statistical tests used were: Student's t test, one-way ANOVA and two-way ANOVA with Tukey post-test, depending on the type of result analysed. All values with p<0.05 were considered statistically significant according to visual demonstration: *p<0.05; **p<0.01; ***p<0.001 and ****p<0.0001.
3 Results

Additional results can be found in the supplemental section.

3.1 Experimental model of obesity

After 8 weeks of a high fat diet, male and female mice presented an average 36% and 27% increase in body weight in comparison to their standard chow fed counterparts, respectively (Fig. 1a). More importantly, mice fed a high fat diet presented a significant increase in perigonadal fat accumulation in comparison to lean mice (Fig. 1c). Metabolic markers like leptin, glucose, triglycerides and total cholesterol were also significantly increased after 8 weeks of a HFD (Fig 1d-g).

3.2 Leucocytes in BAL fluid

Our results confirm that immunization and challenge with OVA induced a significant increase in the total number of leukocytes in BAL fluid, reflected by significantly higher numbers of eosinophils in comparison to sham-immunized animals 24 h after the last OVA challenge (Fig. 2). Standard chow fed female mice presented a significantly higher number of total leukocytes in BAL in comparison to SC male mice (Fig. 2a). This was reflected by a significant increase in BAL macrophages, neutrophils and eosinophils in comparison to female sham-immunized animals and SC OVA males (Fig. 2b-d).

HFD male mice presented a significant increase in total leukocyte numbers in BAL fluid in comparison to male lean OVA and HFD control sham-immunized mice (Fig. 2a). HFD male mice also presented a significantly higher number of eosinophils in BAL fluid in comparison to SC OVA mice and control sham mice (Fig. 2d). In contrast, we did not observe a similar increase in total leukocyte numbers in HFD female mice. In
fact, female HFD mice presented with a significant decrease in the number of leukocytes and eosinophils in BAL fluid in comparison to female SC OVA and male HFD OVA (Fig.2a). Female HFD mice also presented a significant decrease in eosinophils in BAL in comparison to female SC OVA and HFD male OVA group (Fig.2d).

More detail of these data, separated by sex and corrected by weight, can be found in the supplemental section, Figure S1 and S2.

3.3 Leukocytes in lung tissue

Analysis of the number of leukocytes in the lung tissue revealed that male SC OVA mice had a significant accumulation of leukocytes in comparison to SC sham group (Fig.3a). Female SC mice presented a significantly higher number of total leukocytes in response to OVA in comparison to all other groups, following the same trend observed in BAL fluid (Fig.3a). The differential analysis of leukocytes in the lung tissue demonstrated that female SC mice had a significantly higher number of macrophages, neutrophils and eosinophils in comparison to female SC sham and male SC OVA group (Fig.3b-d).

HFD male OVA mice presented a significant increase in total leukocytes in lung tissue in comparison to HFD sham-immunized, but lower when compared to lean OVA mice (Fig.3a). Interestingly, HFD female sham-sensitized animals presented a constitutive and significantly higher number of leukocytes in the lung tissue in comparison to SC sham female mice that was not modified by the sensitization to OVA (Fig.3a). This increased number of leukocytes observed in the lung of HFD female mice is mainly reflected by a significantly higher number of neutrophils, macrophages and eosinophils in the lung in comparison to female SC sham mice and male mice (Fig.3c).
More detail of these data, separated by sex and corrected by weight, can be found in the supplemental section, Figure S3 and S4.

The degree of pulmonary inflammation can be observed in Figure 4 by the analysis of haematoxylin/eosin (Fig. 4a) and Luna staining for eosinophils (Fig 4b).

3.4 Airways remodelling

To clarify whether the differences in leukocyte numbers between sexes would affect airways remodelling parameters, we measured collagen deposition around the airways and TGF-β1 release into BAL fluid. Our results show that in male mice there are no significant differences in collagen deposition around the airways between HFD and SC OVA groups (Fig. 5a-b). However, we observed a significant increase in collagen deposition in HFD OVA female mice when compared to HFD OVA males (Fig. 5a-b). These results were mirrored by the levels of TGF-β1 in BAL fluid, with HFD female OVA animals presenting significantly higher levels of this cytokine in comparison to HFD male OVA mice (Fig. 5c).

3.5 Expression of Adhesion molecules

To better understand the movement of leukocytes between lung tissue and lung lumen, we investigated the effect of a high fat diet on the expression of adhesion molecules associated to the epithelium. We measured the expression of CD103 on BAL cells and the trans-epithelial cell marker EpCam in lung tissue. These results are depicted in Figure 5. Results show a significant increase in EpCam in the lungs of OVA HFD male mice when compared to SC OVA mice (Fig. 6a-b). In contrast, in OVA HFD female mice we observed a reduction in the expression of this adhesion molecule in the lung in
comparison to lean females (Fig. 6a-b). Similarly, the analysis of the expression of CD103 on the surface of BAL cells collected from male HFD mice demonstrated a significant reduction on its expression in comparison to SC mice. Female mice express significantly lower levels of this protein in comparison to males regardless of the diet (Fig. 6c).

3.6 Markers of inflammation

In the last step of our study, we investigated the expression of 2 important markers of inflammation in the lung tissue. We measured the expression of the A20/TNFAIP3 and activated NF-κB (Fig. 7). Independently of the diet, the expression of A20 protein was reduced in the lungs of all OVA groups compared to their respective sham groups. HFD sham animals presented a trend of reduction in the expression of A20 when compared to lean sham animals, but this decrease only reached statistical significance in HFD females (Fig 7a).

The expression of phosphorylated NF-κB in the lung tissue of lean mice revealed that, as expected, OVA-sensitized mice expressed a higher level of this nuclear factor in both male and female lean mice in comparison to sham-immunized mice (Fig.7b). Lean female OVA mice demonstrated a statically higher increase in the activation of activated NF-κB in comparison to sham-sensitized and male mice. However, we did not observe an increase in the expression of this nuclear factor in HFD OVA male or OVA female mice in comparison to their sham-sensitized counterparts (Fig.7b).
4 Discussion

In this study we investigated the interaction between sex and weight in a model of allergic airways inflammation in mice. Using an acute model of lung inflammation induced by OVA we demonstrated sex differences in the response to OVA that are significantly influenced by weight gain. In comparison to males, HFD allergic females exhibited a reduction in the number of leukocytes in BAL when challenged with OVA and, in contrast, an accumulation of these cells in the lung tissue. On the other hand, we also observed that allergic HFD female mice presented with a more robust lung remodelling than allergic HFD males, evidenced by higher deposition of collagen in the airways and higher levels of TGF-β in lung fluid and also a significantly higher levels of IgE in their serum (Supplemental figure 3). We did not observe significant differences in collagen deposition or TGF-β levels between SC and HFD males nor between SC females and SC males suggesting that in our model, airways remodelling is not affected independently by sex or by weight gain but rather by the interaction between these two factors. These findings are in line with what has been described in the literature where several studies have reported a sex difference in obesity and asthma \(^9,23\) with an association often found to be more significant in females than males \(^11,24\). These observations have been frequently linked to the effect of female sex hormones in allergic asthma that have been well documented by us and other authors \(^17,18,25\text{–}29\). In this regard, it has been shown that variations in female sex hormones during puberty, pregnancy or menopause can have a profound effect on asthma symptoms and severity \(^1,2\).

Even though most part of the experimental studies found in the literature regarding allergic inflammation do not make comparisons between sexes, an attenuated eosinophilic response to OVA has been observed in HFD female mice, attributed to a
lower production of eotaxin/CCL11. Other authors have observed similar results in male mice 24h after the last OVA challenge, but not at 48h, suggesting that diet-induced obesity delays the transit of leukocytes through the airway epithelium into the airway lumen by an imbalance in TH2 cytokines. In clinical studies, analysing 131 patients with severe asthma, the authors have reported that airways submucosal eosinophils numbers were higher in obese subjects compared to a lean group, but there was no association between the numbers of eosinophils in sputum or peripheral blood and body mass index, suggesting a delayed transit of these cells through the epithelium into the airways.

Interestingly, the only cell type that was significantly modified by the HFD diet in the lung tissue of OVA males or OVA females were eosinophils. We did not observe significant differences in the number of neutrophils and macrophages between diets within the same sex. Eosinophils are the predominant effector cells in allergic inflammatory diseases and tissue eosinophilia is a hallmark of bronchial asthma and our results confirm this observation. Other experimental studies have described an increased production of eosinophils in the bone marrow of obese mice after the last OVA challenge. Clinically, an increased eosinophilic activity (chemotaxis and adhesion) has been described in atopic asthmatic obese children and adolescent compared to non-obese and non asthmatic volunteers, associated with high serum leptin and TNF-α levels. In our study we did not measure airways inflammation at later time points or a longer period of diet so we cannot rule out the possibility that the attenuated migration of leukocytes to the lung lumen observed in HFD female mice is in fact a delayed response to OVA. However, the fact that we did not observe the same attenuation in HFD males suggests that this result cannot be attributed solely to the diet. Similarly, we cannot rule out significant changes in other cell populations in HFD mice at other time points, as other
authors have described before. More recently, Schroder et al. have also demonstrated that female mice fed a 12-week HFD diet are protected against a lung allergic response to OVA in comparison to lean mice. The authors suggested that this is an effect related to the short term of the diet and not to the obesogenic phenotype per se. The authors reported that HFD female mice presented a reduced airways hyperresponsiveness, airways inflammation, diminished Th1/TH17 but unchanged TH2 differentiation. These results are in line with our observations, where females also presented an attenuated inflammatory response to OVA after an even shorter high fat diet of 8 weeks. However, the authors did not compare their observations to animals fed a HFD for a longer period or to male mice in order to confirm that the attenuation observed in their study is indeed due to the short length of the diet and not to the sex of the animals. This is very important when we consider that in our study, we do not see the same results in male mice fed the same diet and for the same period of time than the females, suggesting that the effect of a shorter diet may be sex dependent.

The inability of leukocytes to cross the epithelial barrier towards the lung lumen when HFD female mice are challenged with an allergen is an indication that an impaired cell recruitment may be occurring. Many of the adhesion molecules involved in the pathology of allergic airways inflammation are expressed in both epithelial and endothelial cells with a few molecules more closely related to the crossing of leukocytes through the epithelium and to its integrity. Among them we can point out EpCam and CD103. CD103 is an integrin protein that is expressed widely on intraepithelial lymphocytes, T cells and regulatory cells. It binds to E-cadherin in the epithelium and it has been demonstrated to be highly expressed in lungs following allergen challenge with either OVA or house dust mite (HDM). The expression of CD103 has also been demonstrated in cells present in BAL fluid and lung homogenates and the absence of
CD103 worsens the migration of leukocytes into the lung and prolongs the response to the allergen, suggesting that CD103 is crucial in regulating the severity of airway inflammation in asthma and may have some important role in the resolution of an allergic inflammatory response\textsuperscript{37}. Our results support these observations as we have demonstrated that CD103 is constitutively inhibited in BAL cells of females, which may explain why female mice present a higher accumulation of inflammatory cells in lung tissue, regardless of the diet. Similarly, we observed a significant inhibition of the expression of CD103 in HFD OVA male mice that may partly explain the worsening of their response to OVA in comparison to lean mice. Further experiments are necessary to determine whether the low expression of this molecule in HFD mice has any effect on the resolution of airways inflammation.

However, CD103 alone does not explain the differences observed between HFD males and females regarding the number of cells in the BAL fluid. Therefore, we investigated the role of the epithelial cell-adhesion molecule (EpCam), an adhesion molecule that is important in the preservation of the integrity of the epithelium. It has also been described as having a stimulatory effect on cell migration in vitro \textsuperscript{39}. EpCam is a transmembrane glycoprotein that has been described in the lower respiratory tract, the trachea, bronchi, bronchioles, and alveoli \textsuperscript{40,41}. In our study, we have found that the expression of EpCam is not significantly different between lean males and lean females exposed to OVA, suggesting that in its expression is not regulated by sex hormones. However, in obesity this dynamic is significantly altered, and we observed an increase in the expression of this protein in the airways of HFD male mice and a significant inhibition in HFD female mice when exposed to the allergen, in comparison to their lean counterparts. These observations agree with what we observed regarding leukocyte migration through the epithelial layer in these animals, suggesting that the interaction
between obesity and sex alters the expression of EpCam in the epithelium of HFD female mice, contributing to their impaired movement of cells to the lung lumen and possibly altering the integrity of their epithelium.

Another important aspect observed in this study is the constitutive accumulation of leukocytes in the lung tissue of female HFD mice that is not significantly altered by sensitization and exposure to OVA. This observation suggests a pre-inflammatory status in the lung of these animals that is not present in the lung of HFD males. In addition, the accumulation of neutrophils in the lung of HFD sham females pointed out to an inflammatory pathway that is not restricted to a TH2 profile. In this regard, data in the literature have suggested that persistent inflammation and airway remodelling in asthma are linked to NF-kb activation. NF-kb is a critical transcription factor that is activated in the airway epithelium of human asthmatics and mice after allergic stimulation, directing high level transcription of many cytokines, adhesion molecules, and other proinflammatory proteins. Inhibitors of NF-kb activation diminish the influx of inflammatory cells and reduce the airway responsiveness induced by the allergen as well the expression of some adhesion molecules. Among endogenous inhibitors of NF-kb, A20 has been suggested to be an important modulator of the inflammatory response in allergic asthma. The A20 ubiquitin protein, also known as TNFAIP3, is a zinc-finger protein that inhibits NF-κB activation by deubiquitinating key signalling pathways downstream of TLR-4, IL-1 and the TNF family of receptors. It has been demonstrated that A20 is an important regulatory molecule in allergic asthma and mRNA levels of A20 measured by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) were shown to be significantly reduced in epithelial cells obtained from subjects with mild or severe asthma when compared with healthy controls, findings confirmed with protein levels. However, despite the evidence of the role of A20 in allergic responses,
little is known regarding its expression in obesity. Some reports in the literature have shown A20 expression is decreased in HFD mice leading to an increase in cardiac dysfunction. In our study we show that the expression of this ubiquitin is decreased in HFD mice in comparison to lean animals, but this was statistically significant only in females, suggesting a pre-inflammatory status in the lung of these animals. Even though we did detect a further decrease in the levels of A20 in OVA HFD females in comparison to sham group, we did not observe an increase in the expression of activated NF-kB in these animals. These results suggest that in obesity, an A20 independent pathways of activation/deactivation of NF-kB may be involved. In this regard, several other endogenous molecules that negatively regulate the activation or activity of NF-kB have been identified. These molecules include CYLD, cyPG15-deoxy-12,14-prostaglandin J2, Foxj1, Twist proteins, and -arrestins.

In summary, our results present evidence that the interaction between sex and weight gain alters the progression of allergic asthma in mice with females developing airways remodelling at a much earlier stage than males. This data may contribute to a better understanding of the clinical differences in the development and severity of allergic asthma observed between men and women in reproductive age.

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6 Conflict of Interest Statement

The authors declare no conflict of interest.
7 References


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Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.
9 Figure legends

Figure 1. Metabolic panel of male and female C57Bl/6 mice fed for 8 weeks with standard chow (SC) or high fat (HFD) diets. Weight was measured weekly (a). At the end of the diet we measured body weight gain (b), the perigonadal fat (c), serum levels of leptin (d), glucose (e), triglycerides (f) and total cholesterol (g). The results are expressed as Mean ± SEM of 10 animals per group. Significant differences among groups were analysed by Student t test. *p<0,05; **p<0,01; ***p<0,001; ****p<0,0001.

Figure 2. Effect of 8 weeks of a standard chow (SC) or high fat (HFD) diets on total and differential leukocytes count in bronchoalveolar lavage (BAL) on male and female C57Bl/6 mice sensitized and challenged with OVA. The intergroup differences on total leukocytes count in BAL of males and females in (a). The differential count for males and for females evidencing the intergroup differences for macrophages (b), neutrophils (c) and eosinophils (d). The results are expressed as Mean ± SEM of 5 animals per group. Significant differences among groups were analysed by two-way ANOVA, followed by Tukey post-test. *p<0,05; **p<0,01; ***p<0,001; ****p<0,0001.

Figure 3. Effect of 8 weeks of a standard chow (SC) or high fat (HFD) diets on total and differential leukocytes count in lung tissue on male and female C57Bl/6 mice sensitized and challenged with OVA. The intergroup differences on total leukocytes count in lung tissue of males and females in (a). The differential count for males and for females evidencing the intergroup differences for macrophages (b), neutrophils (c) and eosinophils (d).
The results are expressed as Mean ± SEM of 5 animals per group. Significant differences among groups were analysed by two-way ANOVA, followed by Tukey post-test. 

*p<0,05; **p<0,01; ***p<0,001; ****p<0,0001.

**Figure 4. Effect of 8 weeks of a standard chow (SC) or high fat (HFD) diets on pulmonary inflammation on male and female mice fed with standard chow (SC) or high fat (HFD) diets for 8 weeks.** In (a) general pulmonary histopathology stained by haematoxylin/eosin. In (b) pulmonary histopathology stained by Luna evidencing eosinophils in red. The photomicrographs refer to the OVA groups (sensitized and challenged to OVA). The scale has 100 μm, the magnification is 20x and the section has 5 μm.

**Figure 5. Measurement of the presence of collagen in the peri-bronchoalveolar space and the presence of fibrotic agent TGF-β1 in male and female mice fed with standard chow (SC) or high fat (HFD) diets for 8 weeks.** In (a) pulmonary histopathology stained by Masson's Trichomial for evidence of collagen fibers (in blue). In (b) transposition of the marked area in histopathology. In (c) production of TGF-β1 measured by ELISA in bronchoalveolar lavage. The photomicrographs refer to the Ova groups (sensitized and challenged to OVA). The scale has 100 μm, the magnification is 20x and the section has 5 μm. The results are expressed as Mean ± SEM of 4-5 animals per group. Significant differences among groups were analysed by two-way ANOVA, followed by Tukey post-test. *p<0,05; **p<0,01; ***p<0,001; ****p<0,0001.
Figure 6. Expression of cell migration molecules in lung tissue and in bronchoalveolar lavage leukocytes from male and female mice fed with standard chow (SC) or high fat (HFD) diets for 8 weeks. In (a), expression of EpCam in the bronchiolar epithelium by immunofluorescence. In (b) transposition of the labelled area into immunofluorescence. In (c) expression of CD103 in immunofluorescence bronchoalveolar lavage leukocytes. The photomicrographs refer to the Ova groups (sensitized and challenged to OVA). The scale has 100 μm, the magnification is 20x and the section has 5 μm. The results are expressed as Mean ± SEM of 4-5 animals per group. Significant differences among groups were analysed by two-way ANOVA, followed by Tukey post-test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Figure 7. Involvement of NF-κB and its repressor, A20 protein, in pulmonary inflammation of male and female mice fed with standard chow (SC) or high fat (HFD) diets for 8 weeks. In (a) and (b) expression of protein A20 (TNFAIP3) and activated NFκB, respectively, in lung tissue measured by immunohistochemistry. The results are expressed as Mean ± SEM of 4-5 animals per group. Significant differences among groups were analysed by two-way ANOVA, followed by Tukey post-test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.