



Modification of lung endoplasmic reticulum genes expression and NF-kB protein levels in obese ovalbumin-sensitized male and female rats

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ABSTRACT

Aims: Previous studies showed a close relationship between obesity and asthma. In this study, we investigated the expression of endoplasmic reticulum (ER) stress genes in the lung tissue of obese ovalbumin (OVA)-sensitized male and female rats.

Main methods: The rats were divided into eight groups ($n = 5$ per group) as follows: female and male rats fed with normal diet (FND and MND, respectively), female and male OVA-sensitized rats fed with normal diet (F-OND and M-OND, respectively), female and male rats fed with high-fat diet (F-HFD and M-HFD, respectively), female and male OVA-sensitized rats fed with high-fat diet (F-OHFD and M-OHFD, respectively). All rats were fed with a high-fat diet or standard pellets for 8 weeks, and for another 4 weeks, they were sensitized by OVA or saline. At the end of the study, lung tissue NF-kB protein level was assessed, and ER stress markers genes expression was determined by Real Time-PCR.

Key finding: OVA-sensitization and diet-induced obesity caused the curve of methacholine concentration-response to shift to the left. In addition, the results indicated that the EC₅₀ (the effective concentration of methacholine generating 50% of peak response) in F-OHFD rats was statistically lower than that of the M-OHFD group ($p < 0.05$). Moreover, the results showed that diet-induced obesity increased the expression of *ATF4*, *ATF6*, *GRP78*, *XBP-1*, and *CHOP* as well as the protein level of NF-kB in this experimental model of asthma, markedly in the F-OHFD group.

Significance: The results suggest that ER stress may be involved in the pathogenesis of asthma observed in obese OVA-sensitized rats, especially in the female animals.

1. Introduction

The endoplasmic reticulum (ER) is a specific organelle that plays an important role in cell homeostasis through biosynthesis, and folding and translational modifications of several proteins [1]. The ER stress process begins when protein synthesis and folding are impaired and increases the accumulation of misfolded and unfolded proteins [2]. Various conditions such as hypoxia, inflammation, increased free fatty acid levels, glucose deprivation, and oxidative stress can impair ER function [3]. As a result of ER stress, an unfolding protein response (UPR) which involves a complex of several signal transduction cascades, is activated in the cells [4]. The 78-kDa glucose-regulated

protein (GRP78) is crucial in initiating the UPR [5]. Three factors namely, RNA-dependent protein kinase (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) can initiate UPR through the GRP78 [5]. In prolonged and severe ER stress, apoptotic pathways are activated through induction of CCAAT/enhancer-binding protein homologous protein (CHOP) [6]. The major actions of IRE1 and PERK are activating the genes involved in the UPR pathway via X-box-binding protein 1 (XBP1) as well as reducing protein translation as a result of activating transcription factor 4 (ATF4) phosphorylation, respectively [7,8]. Interestingly, the three pathways involved in UPR, mediate pro-inflammatory responses through activator protein-1 (AP-1) and NF-KB transcription factors [9]. It was

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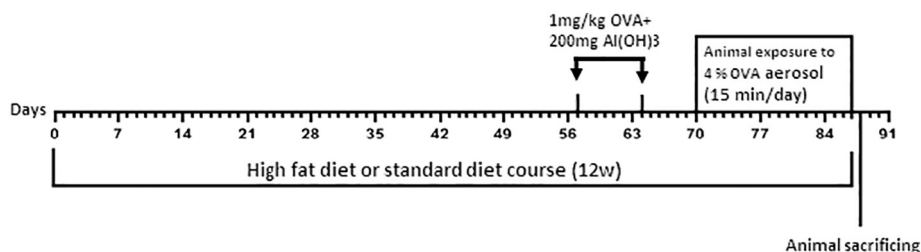


Fig. 1. Method of diet course and animals sensitization.

suggested that ER stress is involved in the pathogenesis of asthma through modification of NF- κ B activity [10].

Asthma is a chronic disease associated with eosinophilia, impaired Th₂ inflammatory response, and allergies, which in many patients, is poorly controlled despite available therapeutic methods [11,12]. According to the recent guidelines, obesity is one of the major factors impairing the management and treatment of asthma [13]. Several studies on patients with asthma showed that “obese asthma” is relatively associated with the following characteristics: resistant to current treatments, increased severity, late-onset nature, increased neutrophils, worse quality of life, and a high prevalence in female [14–16]. Although the precise mechanism associating asthma and obesity has not yet been elucidated, it was revealed that several factors are involved such as mechanical stress, alternations of adipokines, and systemic inflammation [17,18]. Some studies reported many obesity-related disorders, including hyperlipidemia, insulin sensitivity, hyperglycemia, hypertension, elevated pro-inflammatory factors, and central obesity [11]. The airway hyperresponsiveness in obese ovalbumin (OVA)-sensitized rats was demonstrated compared to the lean OVA-sensitized rats, which was more pronounced observed in female animals [19,20]. In obesity-related asthma, some important features are observed in AHR, such as the altered expression and secretion of interleukins, adipokines, transcription factors, and some microRNAs as well as insulin sensitivity, hyperlipidemia [21–25].

Given the association between inflammatory responses and ER stress, changes in ER stress may be implicated in the pathogenesis of obesity-related asthma. However, there is no report on the role of ER in obese asthma patients. The present study aimed at investigating the changes in the expression level of ER stress markers in obese OVA-sensitized rats compared to the lean OVA-sensitized animals. In addition, possible sex differences affecting ER stress markers as well as NF- κ B protein levels in obese OVA-sensitized rats were evaluated.

2. Materials and methods

2.1. Animals and diets

In the current experiment, a total of 40 female and male Wistar rats (150 ± 10 g) were prepared from the Animal House of Tabriz University of Medical Sciences. All animals were maintained under controlled conditions (i.e. 12 h:12 h light-dark cycle at 22 ± 2 °C). During the experimental period, animals had free access to food and water.

The estrous cycle in female Wistar rats was determined based on the vaginal smear cytology as described previously [25]. Briefly, vaginal smears were taken by slowly injecting 0.3 ml into the animal's vagina. The infused fluid was applied with two to three drops for smear preparation. Menstrual cycles were determined using the light microscopy and female animals with estrous cycle were selected for subsequent evaluations. The estrous phase was characterized by presence of high cornified cells but absence of leukocytes.

2.2. Preparing the diet

The rats were divided into eight groups ($n = 5$ per group) as follows: female and male rats fed with the normal diet (FND and MND, respectively), female and male OVA-sensitized rats fed with the normal diet (F-OND and M-OND, respectively), female and male rats fed with high-fat diet (F-HFD and M-HFD, respectively), and female and male OVA-sensitized rats fed with high-fat diet (F-OHFD and M-OHFD, respectively).

Based on previous studies, high fat diet (HFD) was used to induce obesity. Briefly, diets in HFD groups (HFD and O-HFD) had 42% energy from fat, 19% from protein, and 39% from carbohydrate. In the normal diet groups, the animals were fed with standard chewing food (11% energy from fat, 28% from protein, and 61% from carbohydrate) [21].

All rats were fed for 8 weeks with a high-fat diet or standard pellets, and for another 4 weeks, they were sensitized with OVA or saline while receiving the assigned diet.

2.3. Ovalbumin sensitization

OVA sensitization was performed using intraperitoneal injection of 1 mg OVA + 200 mg Al (OH)₃ (Sigma) on days 1 and 8. While the animals were breathing normally in a closed chamber (dimensions: 30 × 20 × 20 cm), the animals were exposed to 4% OVA aerosol for 15 min from day 14 to 31. The nebulizer used to create the OVA aerosol was CX3 (Omron Health Care Europe B.V., the Netherlands) [26,27]. The rats of the ND and HFD groups were treated similarly with saline instead of OVA solution (Fig. 1). Animal handling was approved by the Ethical Committee of Ardabil University of Medical Sciences, Ardabil, Iran (IR.ARUMS.REC.1397.241).

2.4. Determination of tracheal responsiveness to methacholine

The rats were anesthetized by injecting a combination of ketamine (50 mg/kg) and xylazine (3 mg/kg) intraperitoneally. After opening the chests, approximately 2 cm from the tracheae was isolated and immediately put into a cool and oxygenated solution. The tracheae were divided into several 5-mm segments (five to six ring cartilages). Finally, each tracheal segment was immediately placed between two horizontal stainless steel hooks suspended in a 20-ml organ bath (Schuler organ bath type 809, March-Hugstetten, Germany) containing Krebs-Henseleit solution with the following composition (mM): NaCl 120, NaHCO₃ 25, MgSO₄ 0.5, KH₂PO₄ 1.2, KCl 4.72, CaCl₂ 2.5, and dextrose 11. Krebs solution was maintained at 37 ± 1 °C and gassed with 95% O₂ and 5% CO₂. The initial tension was 1 g throughout the experiment, and the equilibrium period was 60 min during which, the bath solution was replaced with fresh Krebs solution every 15 min. Responses were measured using an isometric transducer (ADInstruments, Spain) with the sensitivity range of 0–25 g, amplified with an amplifier (ML/118 Quadri Bridge Amp, March-Hugstetten, Germany), and recorded by a PowerLab (ML-750, four-channel recorder, March-Hugstetten, Germany). Finally, the following criteria were evaluated: cumulative log concentration-response curve of methacholine hydrochloride (Sigma Chemical Ltd., UK), EC₅₀ (as the effective concentration of

Table 1
Primer set list for mRNAs.

Gene name	Primer sequence
ATF4	Forward primer: GCTATGGATGGGTTGGTCAG Reverse primer: AAGCTCATCTGGCATGGTTTC
ATF6	Forward primer: TGGATTTGATGCCTTGGGAGT Reverse primer: AGGAACGTGCTGAGTTGAAGA
GRP78	Forward primer: CTGGTCTGCTTGATGTGTGT Reverse primer: TTGTCTTTTGCAGGGGTCG
XBP1	Forward primer: AAGTGGTGGATTGGAAGAAGA Reverse primer: CTCTTCAGTAACCAGGGCATT
CHOP	Forward primer: GGCACCTATATCTCATCCCCA Reverse primer: CCACTCTGTTTCCGTTTCCTAG
GAPDH	Forward primer: TTGCCATCAACGACCCCTTCA Reverse primer: AGCACCAGCATCACCCCATTT

methacholine generating 50% of peak response), and contractility response to 10 μ M methacholine (as the g force/mg tissue weight (gF/TW)).

In addition, to assess weight changes and obesity model by high-fat diet, obesity indices in rodents, including Lee index, percentage of body weight changes, and percentage of body fat were used [25].

2.5. Determination of lung tissue NF- κ B protein levels

To measure the levels of NF- κ B protein in the lung tissue of the study groups, the animals were sacrificed and their isolated lung were stored at -70°C until measurement.

2.6. Real-time polymerase chain reaction

For measurement of *ATF4*, *ATF6*, *GRP78*, *XBP1*, and *CHOP* mRNA expression levels, we used the method of a previous study [23]. Table 1 shows the locked nucleic acid (LNA) forward and reverse primer sets (Exiqon). The PCR products were normalized against *GAPDH*. Results are expressed as fold change versus the relevant controls.

2.7. Statistical analysis

In the current study, results are reported as mean $\pm$ SD. Comparison of different groups were made using ANOVA and Tukey-Kramer post hoc test. To compare differences between the two sexes, the independent two-sample *t*-test was employed. A $P < 0.05$ was considered significant. The correlation analysis was performed by using Pearson's correlation coefficient test.

3. Results

3.1. Body weight

In both sexes, weight changes were larger in the high-fat diet groups (HFD and O-HFD) than the normal diet groups (ND and O-ND) (Fig. 2a and b). In addition, obesity indices in both sexes (percentage of body weight change, Lee index, and percentage of body fat) in the high-fat diet groups were significantly higher than the normal diet groups (Table 2). Interestingly, obesity indices were higher in male rats compared to the female rats; however, the percentage of weight changes in the HFD group in comparison to the ND group, was higher in the female rats than the male rats (25 vs. 11%, respectively).

3.2. Tracheal response to methacholine

In both sexes, the methacholine concentration-response curves in the OVA-treated groups (O-ND and O-HFD) was shifted to the left compared to the non-sensitized groups (ND and HFD), and the left-ward shift was more obvious in the obese OVA-treated groups (Fig. 3a and b).

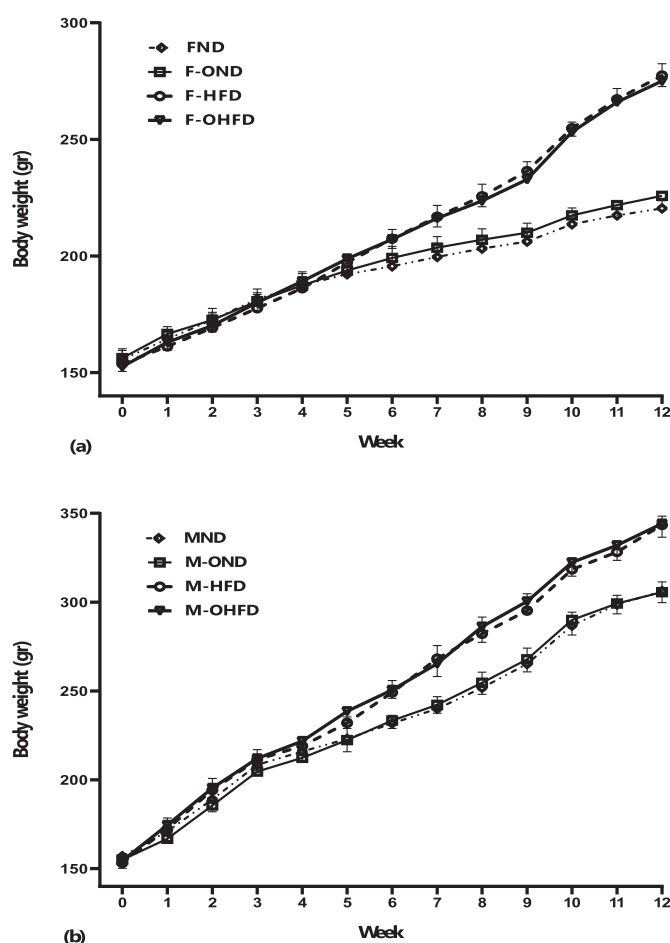


Fig. 2. Weight courses in different groups. Values are expressed as mean $\pm$ SD in a) female, and b) male groups. FND: female normal diet group, MND: male normal diet group, F-OND: female OVA-sensitized with a normal diet, M-OND: male OVA-sensitized with a normal diet, F-HFD: female high-fat diet group, M-HFD: male high-fat diet group, F-OHFD: female OVA-sensitized with a high-fat diet, M-OHFD: male OVA-sensitized with a high-fat diet. For each group, $n = 5$.

Table 2
Obesity indexes in experimental groups.

Groups	Percentage of body weight change (%)	Lee index (mg/mm)	Percentage of body fat (%)
ND			
F	41.73 $\pm$ 4.21	281.71 $\pm$ 1.79	4.77 $\pm$ 1.23
M	95.17 $\pm$ 6.00	296.55 $\pm$ 3.45	15.01 $\pm$ 2.38
P-value	***	***	***
O-ND			
F	44.61 $\pm$ 3.49	282.16 $\pm$ 4.93	5.08 $\pm$ 3.40
M	97.16 $\pm$ 2.97	297.68 $\pm$ 2.87	15.79 $\pm$ 1.98
P-value	***	**	**
HFD			
F	80.41 $\pm$ 5.05	304.51 $\pm$ 3.57	20.50 $\pm$ 2.46
M	124.23 $\pm$ 5.98	309.56 $\pm$ 3.26	24.05 $\pm$ 2.25
P-value	***	*	*
O-HFD			
F	80.22 $\pm$ 1.69	301.93 $\pm$ 3.05	18.72 $\pm$ 2.11
M	122.98 $\pm$ 4.57	310.20 $\pm$ 4.89	24.42 $\pm$ 3.37
P-value	***	*	*

Values are represented as mean $\pm$ SD. F: female, M: male, ND: normal diet group, O-ND: OVA-sensitized with a normal diet, HFD: high-fat diet group, O-HFD: OVA-sensitized with a high-fat diet. Differences between gender; ns: no significant. For each group, $n = 5$.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

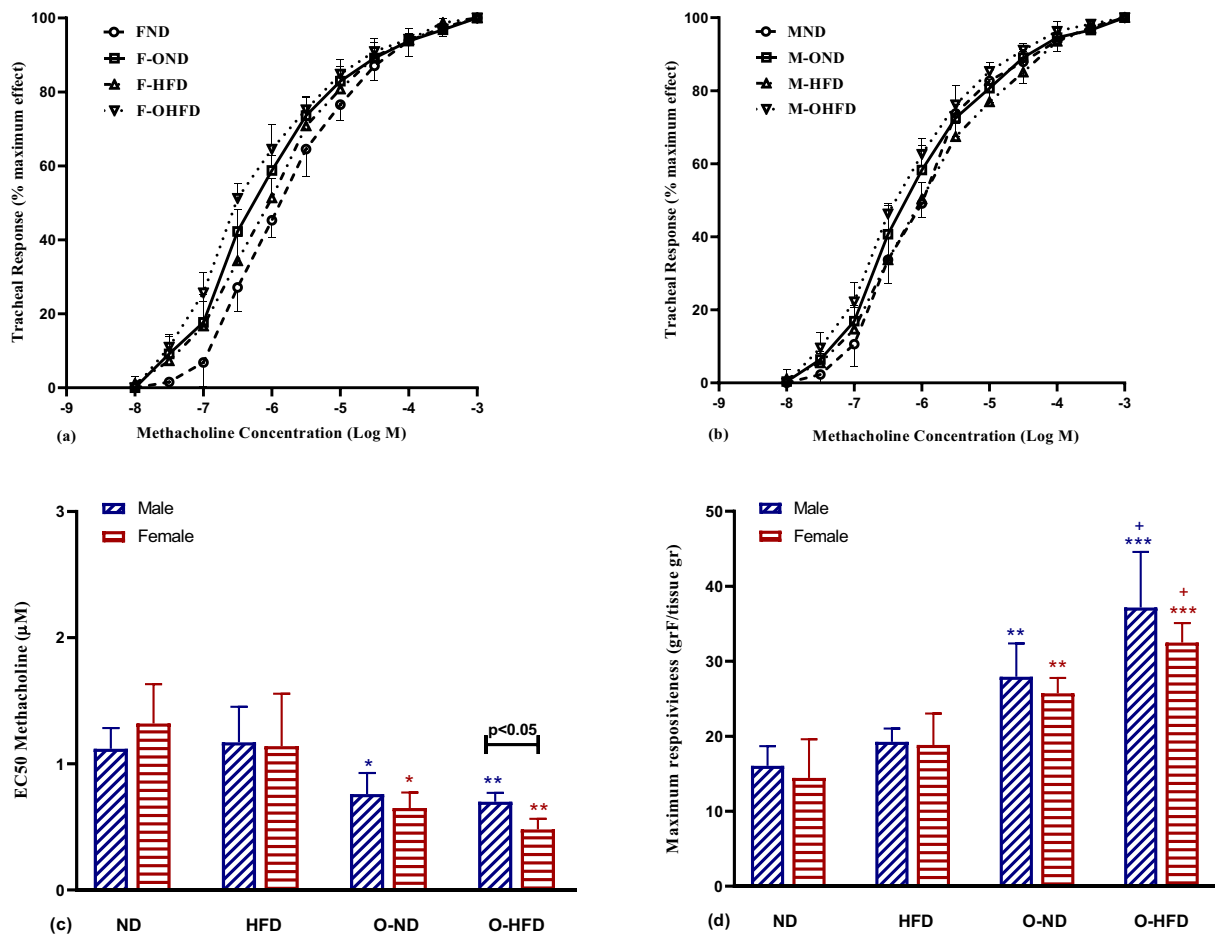


Fig. 3. Cumulative log concentration-response curves of methacholine-induced contraction of the isolated trachea in a) female and b) male Wistar rats; the mean + SD of c) tracheal response to methacholine (EC_{50} , the effective concentration of methacholine, causing 50% of maximum response) in female (red) and EC_{50} in male (blue) rats; d) tracheal contractility to methacholine (gr force/tissue gr) (contractility response to 1 mM methacholine) in female (red) and male (blue) rats. ND: normal diet group. Abbreviations: same as Fig. 2.

In both sexes, Statistical differences between high-fat diet group with lean and obese OVA-sensitized groups in both sexes are indicated with: *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$. Statistical difference between normal diet OVA-sensitized vs. high-fat diet OVA-sensitized rats in both sexes: +: $p < 0.05$.

The blue column and symbols indicate group differences in male rats. The red column and symbols show group differences in female rats. Black symbols indicate sex differences between males and females in each group. ($n = 5$ per group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Moreover, EC_{50} was significantly lower in both sexes in the OVA-treated groups (O-ND and O-HFD) than the non-OVA-treated groups (ND and HFD), which was more marked in the diet-induced obesity (DIO)-OVA-sensitized groups. Interestingly, EC_{50} was significantly lower in the obese OVA-sensitized female rats (0.48 ± 0.08) than the obese OVA-sensitized male rats (0.70 ± 0.07 , $p < 0.01$) (Fig. 3c).

Based on the contractility results, it was found that in both sexes, the level of gF/TW was significantly higher in the OVA-treated groups (O-ND and O-HFD) than the non-OVA-treated groups (ND and HFD). In addition, in both sexes, gF/TW was higher in the DIO-OVA-sensitized group compared to the normal diet OVA-sensitized group (Fig. 3d). Although the contractility in the OVA-sensitized male rats was higher than that of the OVA-sensitized female rats, the difference was not statistically significant (Fig. 3d).

3.3. ER stress genes expression levels

In the female rats, the results showed that the expression levels of *ATF4*, *ATF6*, *GRP78*, *XBPI*, and *CHOP* were higher in the OVA-treated groups (O-ND and O-HFD) compared to the non-OVA-treated groups (Fig. 4a–e). Furthermore, in female rats, it was found that the obese-OVA-sensitized rats compared with lean OVA-sensitized rats, showed

high levels of expression of *ATF6* (6.76 ± 1.42 vs. 4.30 ± 0.56 , $p < 0.01$), *GRP78* (5.23 ± 1.02 vs. 3.29 ± 1.17 , $p < 0.01$), *XBPI* (5.67 ± 1.03 vs. 3.59 ± 0.96 , $p < 0.01$), and *CHOP* (7.96 ± 0.64 vs. 5.34 ± 0.59 , $p < 0.001$) genes (Fig. 4).

Moreover, in males, it was found that the expression levels of *ATF4*, *ATF6*, *GRP78*, and *CHOP* were higher in the OVA-sensitized groups (O-ND and O-HFD) in comparison to the non-OVA-sensitized rats (ND and HFD) (Fig. 4a–e). The expression level of *XBPI* in male rats, only in O-HFD group, was higher than the non-OVA-sensitized groups (Fig. 4d). The results also indicated that the expression levels of *ATF6* and *CHOP* genes were higher in the O-HFD group (6.04 ± 0.49 and 6.18 ± 0.72 , respectively) than the O-ND group (4.02 ± 0.63 and 4.04 ± 0.39 , respectively) ($p < 0.001$ for both).

Comparing ER stress genes expression levels of the two sexes revealed that the expression level of *GRP78*, *XBPI*, and *CHOP* in the O-HFD group was higher in female rats compared to the male rats. In addition, in O-ND group, the expression level of *XBPI* and *CHOP* was higher in female rats compared to the male rats (Fig. 4d and e).

3.4. Lung tissue NF- κ B protein levels

In both sexes, lung tissue NF- κ B protein levels were higher in the

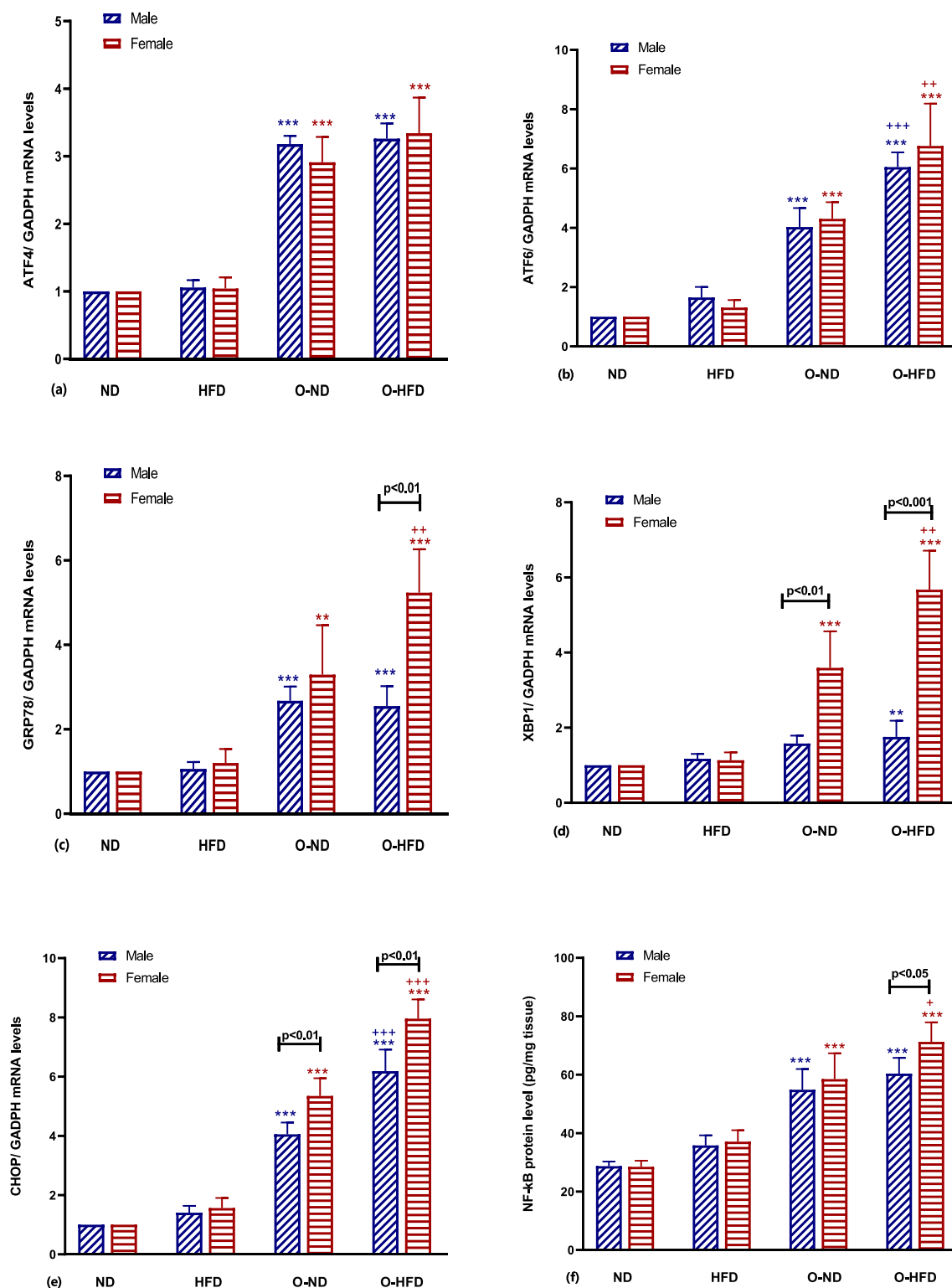


Fig. 4. ER stress markers gene expression level and NF-κB lung tissue protein level. Mean + SD of a) ATF4, b) ATF6, c) GRP78, d) XBP1, and e) CHOP expression levels in female (red) and male rats (blue). Also, f) NF-κB lung tissue protein level in female (red) and male rats (blue). Abbreviations: same as Fig. 2. In both sexes, statistical differences between the HFD and other groups; **: $p < 0.01$, ***: $p < 0.001$. Statistical difference between normal diet OVA-sensitized vs. high fat diet OVA-sensitized in both sexes: +: $p < 0.05$, ++: $p < 0.01$, +++: $p < 0.001$. For each group, $n = 5$.

The blue column and symbols indicate group differences in male rats. The red column and symbols show group differences in female rats. Black symbols indicate sex differences between males and females in each group. ($n = 5$ per group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Pearson correlation analysis of variables of study and EC50 levels.

Variables	EC50			
	Female		Male	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
Percentage of body weight changes (%)	−0.167	0.482	−0.039	0.872
Lee index (mg/mm)	−0.312	0.181	−0.053	0.823
Percentage of body fat (%)	−0.312	0.181	−0.053	0.823
ATF4	−0.784	0.000	−0.814	0.000
ATF6	−0.705	0.000	−0.775	0.000
GRP78	−0.708	0.000	−0.776	0.000
XBP1	−0.693	0.001	−0.647	0.000
CHOP	−0.768	0.000	−0.772	0.000
NF-κB	−0.742	0.000	−0.726	0.000

OVA-sensitized groups (O-ND and O-HFD) in comparison to the non-OVA-sensitized groups (ND and HFD). In the female rats, lung tissue levels of NF-κB protein were higher in the O-HFD group (71.23 ± 6.70) compared to the O-ND group (58.50 ± 8.80 , $p < 0.05$); however, no significant difference was observed in male rats (Fig. 4f). In addition, in the O-HFD group, NF-κB protein levels in female (71.23 ± 6.70) rats were higher than male rats (60.37 ± 5.41 , $p < 0.05$), but in the O-ND group, no significant difference in NF-κB level was observed between males and females.

3.5. The correlation between study variables and EC50

In both sexes, the results showed a negative association between EC50 and NF-κB protein level, and ATF4, ATF6, GRP78, XBP1, and CHOP gene expression levels (Table 3).

4. Discussion

The obtained results showed that tracheal responsiveness to methacholine in both sexes was more evident in the OVA-sensitized groups, especially in obese groups. Furthermore, the expression level of ER stress markers in both sexes was higher in obese OVA-treated groups. Moreover, it was found that the expression levels of ER stress markers as well as NF-κB lung tissue protein levels were higher in the obese OVA-sensitized female rats compared to the male rats.

The increased tracheal responsiveness to methacholine in the OVA-sensitized male and female rats, was in line with the results of previous studies [21,25]. This finding also confirmed previous data concerning AHR development in OVA-sensitized mice [19]. In addition, the higher airway hyperresponsiveness in obese OVA-sensitized female rats in comparison to the male rats, was consistent with the results of previous studies [19,25].

Asthma is a chronic airway disease, which is mainly associated with allergic factors and occurs due to the dysregulation of Th₂ inflammatory responses and eosinophilia [11,28]. Various studies showed that ER plays an important role in the pathogenesis of asthma. Overexpression of ER stress markers such as GRP78, CHOP, ATF6, XBP-1, and p-eIF2 in the lung tissue of OVA-sensitized mice, was previously reported [10,29]. In the present study, our results showed that in the lean OVA-sensitized male and female rats compared to the non-OVA-sensitized animals, the expression levels of ATF4, ATF6, GRP78, XBP-1 and CHOP were significantly increased. The results of our study were consistent with previous studies which indicated that the increased airway responsiveness in OVA-sensitized mice may be due to the changes in ER stress markers [10]. In BAL fluid samples collected from asthmatic patients, increased levels of ER stress markers were observed [30].

Furthermore, our results showed that some ER stress markers (ATF6 and CHOP) were significantly increased in obese OVA-sensitized male and female rats in comparison to the lean OVA-sensitized groups.

Interestingly, the expression levels of GRP78, XBP-1 and CHOP genes in lung tissue of obese OVA-sensitized female rats, were higher than obese OVA-sensitized male rats. In fact, our results suggest that in both sexes, ER stress was more severe in obese OVA-sensitized rats, especially in female rats.

Animal studies showed that conditions, such as obesity, chronic inflammation, and oxidative stress can produce ER stress and activate UPR [30]. Also, ER stress was reported to stimulate production of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor (TNF)-α, and interleukin 1 beta (IL-1β) through several cellular pathways like c-Jun N-terminal kinases (JNK) and IKB kinase (IKK) [31]. Therefore, ER can be regarded as one of the main sites to notice obesity and inflammation. ER stress comprises a series of molecular cascades consisting of enzymes and transcription factors, to maintain cellular homeostasis. The key factor in initiating UPR is a protein called GRP78 that is associated with the protein folding process [32]. In fact, increased expression of GRP78 indicates enhanced misfolded protein level in the ER and is a marker for ER stress assay [32]. Animal studies found an increase in GRP78 and CHOP levels as well as neutrophil counts in the lung tissue of the OVA-lipopolysaccharides (LPS)-treated mice [10].

One of the cascade molecules of GRP78 is IRE1 with various functions, such as XBP1 mRNA splicing and activation of the NF-κB transcription pathway by degradation of IκBα [5,33]. A study by Boden et al. showed activation of the UPR pathway and elevated levels of XBP1 in subcutaneous adipose tissue of obese human subjects [34]. The IRE1/XBP1 axis is an important pathway in maintaining a variety of physiological processes and plays a dual role in cell death and stress adaptation. The IRE1/XBP1 axis activity is dependent on the duration of stress and cell types and can be programmed for cell death through activation of the CHOP pathway or for adaptation to stress [35].

When GRP78 is activated in the UPR pathway, its other branch, ATF6, is transferred from the ER to the Golgi apparatus. By inducing transcription of enzymes and chaperones, ATF6 promotes ER folding and homeostasis [3]. In addition, ATF6 also activates CHOP and XBP1 genes [3]. It was also shown that ATF6 can activate the NF-κB pathway by cytotoxins-induced ER stress pathway [9].

In severe or chronic ER stress, apoptotic processes begin through CHOP and other mechanisms [5]. CHOP gene expression was shown to be increased in both lungs and BAL samples of asthmatic patients [10]. In the current study, there was a significant increase in both sexes in the expression levels of GRP78, XBP1, and CHOP in lung tissue of obese OVA-sensitized rats. Interestingly, the increased expression of ER stress genes was significantly higher in obese OVA-sensitized female rats than male rats.

The results of our study also showed that the protein levels of NF-κB in lung tissue, were higher in obese OVA-sensitized female rats compared to other groups. Previous studies reported that ER stress is capable of activating NF-κB signaling pathways, leading to a significant increase in expression and production of pro-inflammatory cytokines [36]. The pathophysiological changes of lung tissue ATF6, XBP1 and PERK are associated with the NF-κB pathway [36]. In fact, it was suggested that ER stress exacerbates inflammatory responses through activation of NF-κB.

Based on the results of our study, it can be inferred that an increase in airway responsiveness in obese OVA-sensitized rats in both sexes, especially in female rats, could be due to changes in the ER stress pathway. However, the authors cannot rule out the role of other factors, such as adipocytokines in enhancing airway responsiveness. In previous studies, we showed that protein levels of some adipocytokines, like visfatin were increased in the lung tissue of obese OVA-sensitized male and female rats, which was highly correlated with tracheal responsiveness [22,25]. Therefore, along with changes in ER stress markers, adipokine-related changes may have an impact on the airway responsiveness in obesity-related asthma, which requires further studies.

Although exact mechanisms through which sex differences affect ER

stress markers has not yet known, the role of sex differences in the expression of ER stress genes was shown in some studies. In patients with heart diseases, ER stress markers were higher in male patients than women [37]. Based on the results of our previous study as well as the results of the current study, it can be concluded that sex differences are possibly associated with variations in the expression levels of ER stress markers; however, further studies are required to confirm these findings.

While our study results showed alterations in the expression of ER stress markers in the lung tissue of obese OVA-sensitized rats as well as sex differences in this pathway, the study did have some limitations. First, in the current model, we used whole lung tissue to study gene expression. Although different compartments of the lung such as vascular or endothelium were shown to affect disease severity or sex differences, our study was not able to detect these differences. Indeed, it was shown that the differential expression of some genes is related to the hematological system, cell-cell interactions, and connective tissue disorders. The second limitation was the lack of examination of the levels of protein of ER stress genes in the present study. Therefore, further studies are needed to determine the factors influencing the post-translational stage of gene expression.

In conclusion, the results showed that ER stress markers as well as lung tissue NF- κ B protein levels were associated with AHR in obese OVA-sensitized male and female rats, especially in female rats. Therefore, ER stress markers can be considered pathological and therapeutic agents in obesity-related asthma.

Authorship statement

Mohammad Reza Aslani: Literature search, Proposal writing, Data collection, Analysis of data, Interpretation of data, Manuscript preparation, and Review of the manuscript.

Hassan Ghobadi, Hamdollah Panahpour, Mahdi Ahmadi, Majid Khaksar, and Morteza Heidarzadeh: Proposal writing, Data collection and Analysis, Draft preparation, and Review of the manuscript.

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Declaration of competing interest

The authors have declared that there is no conflict of interest.

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