ORGINAL ARTICLE
Iran J Allergy Asthma Immunol
February 2019; 18(1):48-61.

Effect of High-fat Diet on Tracheal Responsiveness to Methacholine and Insulin Resistance Index in Ovalbumin-sensitized Male and Female Rats

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Received: 25 January 2018; Received in revised form: 1 July 2018; Accepted: 4 July 2018

ABSTRACT

Epidemiological and clinical studies have demonstrated a close association between obesity and asthma. The current study investigated the effect of high-fat diet on tracheal responsiveness to methacholine and insulin resistance in ovalbumin (OVA) sensitized male and female rats.

The rats were divided into eight groups (n=6 per group): female with the normal diet (F+ND), male with the normal diet (M+ND), female OVA-sensitized with the normal diet (F+SND), male OVA-sensitized with the normal diet (M+SND), female with high-fat diet (F+HFD), male with high-fat diet (M+HFD), female OVA-sensitized with high-fat diet (F+SHFD), and male OVA-sensitized with high-fat diet (M+SHFD). All rats were fed for 8 weeks with high-fat diet or standard pelts, and for another 4 weeks, they were sensitized with OVA or saline. At the end of the study, the tracheal responsiveness to methacholine, serum insulin, and blood glucose levels was measured. Also, insulin resistance indexes were determined.

OVA-sensitization and diet-induced obesity caused the curve of methacholine concentration response to shifting to the left. In addition, results indicated that the EC_{50} (the effective concentration of methacholine generating 50% of peak response) in F+SHFD rats was statistically lower than M+SHFD group ($p<0.05$). Moreover, insulin resistance was higher in the F+SHFD than the M+SHFD group ($p<0.001$).

These results suggest that insulin resistance and metabolic syndrome may be involved in the pathogenesis of obesity associated with OVA-sensitized rats condition, especially in female animals.

Keywords: Airway hyper-responsiveness; Asthma; Insulin-resistance; Obesity; Wistar rats

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INTRODUCTION

Asthma is a chronic disease worldwide which is still not significantly controllable despite progress in its treatment.[1] Obesity is one of the factors that may lead to the impairment of the control of asthma symptoms.[2] Epidemiological studies reported a relationship between asthma and obesity.[3,4] Until now, the exact mechanism for this relationship has not been completely specified. The results regarding the association between obesity and increased airway hyper-responsiveness (AHR) in asthma are very controversial. While some studies found no such association,[5,6] others have shown relationships with variable outcomes. In some studies, AHR was reported only in females,[7,8] while in some others only in males,[9,10] and in some studies in both sexes.[11,12] In the animal model of diet-induced obesity, it is revealed that obesity leads to AHR in ovalbumin (OVA) sensitized mice when compared with control mice.[13] Interestingly, obesity independent of asthma is capable of inducing AHR in animal studies.[13]

Studies examining the link between asthma and obesity suggested an interaction between obesity, metabolic syndrome, systemic inflammation, and asthma.[14] Indeed, it has been shown that the markers of metabolic syndrome such as hypertension, hyperlipidemia, and insulin resistance can be associated with obesity and asthma.[15] Several human studies have reported that increased insulin level and insulin resistance were related to reduced lung function and increased AHR through several mechanisms such as airway smooth muscle proliferation and epithelial damage.[16] In this regard, metabolic syndrome has been shown to worsen asthma control and may have an impact on the treatment process in patients with asthma,[17] especially women.[18]

On the other hand, several human and animal models of allergic airway disease have revealed sexual dimorphism in allergic conditions such as asthma.[19,20] Female mice have been identified a higher susceptibility for airway allergic disease than male mice.[19] Among factors influencing the gender difference, the lower protection against inflammatory stimuli in female mice compared to male mice can be noted.[19] Although there were some studies on the role of obesity and metabolic syndrome in asthma, few studies have examined sexual dimorphism in obesity with asthma. Therefore, the present study investigated the effect of high-fat diet on tracheal responsiveness to methacholine and insulin resistance in OVA-sensitized male and female rats.

MATERIALS AND METHODS

Animals and Diets

The present study was performed on forty-eight male and female Wistar rats weighing approximately 160 g, obtained from the Animal House of the Tabriz University of Medical Sciences, Tabriz, Iran. The animals cared in cages under controlled conditions of 12:12 h light-dark cycle and a 22±2^{o}C. Food and water were accessible *ad libitum* during the accommodation and experimental period.

In female animals, vaginal smear cytology was used to determine the phases of the estrus cycle.[21] Based on this method, the ratio of three different types of cells in the vaginal smear is determined which included cornified cells, epithelial cells, and leukocytes. To prepare vaginal smear, approximately 0.3 ml of saline was slowly injected into the animal's vagina by a Transferpette sampler. Then, 1-2 drops of the injected fluid were used to make a smear. Smear test was performed using light microscopy. The selected of female rats for later stages of the study was based on the menstrual cycle during the estrous period. The menstrual cycle is characterized by high cornified cells than epithelial cells and the absence of leukocytes

Diet and OVA-Sensitization

All animals were randomly subclassified into eight groups (6 rats per group): female with the normal diet (F+ND), male with the normal diet (M+ND), female OVA-sensitized with the normal diet (F+SND), male OVA-sensitized with the normal diet (M+SND), female with high-fat diet (F+HFD), male with high-fat diet (M+HFD), female OVA-sensitized with high-fat diet (F+SHFD), and male OVA-sensitized with high-fat diet (M+SHFD).

In order to diet-induced obesity (DIO) model, we used the previous studies model.[22,23] Briefly, animals in the DIO groups (HFD and S+HFD) received high-fat diets (42% energy from fat, 19% energy from protein, and 39% energy from carbohydrate), and in the normal diet groups (C+ND and S+ND) animals received standard rat chow (11% energy from fat, 28% energy from protein, and 61% energy from carbohydrate).

Rats were fed for 8 weeks in accordance with the

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abovementioned diet and were used for the induction of sensitization with ovalbumin in S+ND and S+HFD groups (male and female) from the following protocol. The experiment continued for four weeks for the OVA-sensitized group with the previous regime.

Animal Sensitization

The rats in sensitized groups (S+ND and S+HFD) were intraperitoneally sensitized with 1 mg OVA (Sigma grade 5) dissolved in 0.9% saline and 200 mg aluminum hydroxide (Sigma, Chemical Ltd, UK) on days 1 and 8. Animals from day 14, were then exposed to aerosol particles of 4% OVA for 17–19 days produced by a nebulizer (CX3, Omron Health Care Europe B.V., the Netherlands) 15 min/daily in a closed chamber (dimensions: 30×20×20 cm).[24-26] The rats of the normal diet (ND) and high-fat diet (HFD) groups were treated similarly with saline instead of OVA solution.

The Assessment of Tracheal Responsiveness to Administration of Methacholine

In each experiment, a cumulative log concentration-response curve of methacholine hydrochloride (Sigma Chemical Ltd, UK) was obtained in each tracheal segment by adding consecutive concentrations (10^{-8} to 10^{-3} mM) every 2 min. To plot the curve, the percentage of contraction of the tracheal smooth muscle caused by each methacholine concentration in proportion to the maximum contraction acquired by its final concentration was plotted against log concentration of methacholine. Then, EC_{50} as the effective concentration of methacholine generating 50% of peak response was measured from the methacholine response curve in each experiment using 50% of maximum response in the Y-axis and measuring the dose of methacholine causing this response in the X-axis. The contractility response to 10µM methacholine was also determined as the magnitude of contraction. At the end of the experiments, the tissues were weighed. Afterward, the contraction force induced by methacholine (at the plateau level) was calculated to express as the gr force/mg tissue weight (gF/TW) to compare the contraction activity of this spasmogen between groups.

Body Weight

At the end of the study, after induction of anesthesia, the animals were weighed and measured for their naso-anal length, i.e. the distance between nose and anus. To calculate the obesity indices in rodents, determined items such as the final body weight, the percentage of body fat (BF%), and Lee index. The following formula indicates the calculation of all obesity indices:

Percentage of body weight changes (%)=[(final weight-initial weight)/initial weight] ×100.[27]

Lee index (mg/mm)=Final weight$^{0.33}$ / naso-anal length.[27]

Percentage of body fat (%)=0.73 (Lee index-280.8).[27]

The study was approved by the Ethical Committee of Ardabil University of Medical Sciences, Ardabil, Iran (N. IR.ARUMS.REC.1396.98).

Fasting Blood Glucose, Oral Glucose Tolerance Test, Serum Insulin, Insulin Resistance, and Insulin Sensitivity

At the end of the study, before and after the glucose tolerance test was done following oral glucose administration (1 g/ kg body weight), fasting/basal serum glucose and serum insulin levels were determined. Overnight (12h) fasted rats were orally administered with dextrose anhydrous by gavage. Peripheral blood samples were taken from the tip of the tails. Blood glucose levels were measured 0, 30, 60, 90, and 120 min after dextrose administration using a digital glucometer (Gluco Sure, Star, Taiwan). For serum insulin measurement, after blood samples clotted at room temperature, serum samples were extracted and stored at -70°C until analysis. The serum insulin concentrations were measured using a rat insulin commercial kit (Bioseps, Co. Ltd, China). Insulin resistance estimation was performed using the homeostasis model assessment method, HOMA-IR, and was calculated using the following formula:

Blood glucose (mg/dL)×fasting plasma insulin (IU mg/L in the fasting state) divided by 405.[28]

Also, to calculate insulin sensitivity, the QUICKI index was used with the following formula:

QUICKI=1/[log (fasting insulin, µU/mL)+log (fasting glucose, mg/dL)].[28]

Statistical Analysis

Results were represented as mean ±SEM. Analysis of variance (ANOVA) was used for compared between different groups with Tukey-Kramer post hoc test. The independent two-sample t-test was utilized for the

analysis of the continuous variable in two sex groups. $p<0.05$ was considered significant. The correlation between glucose levels and study parameters was assessed with Pearson's correlation coefficient. All statistical analysis was performed with SPSS Statistics for Windows, version 16.0 (SPSS Inc., Chicago, Ill., USA).

RESULTS

Body Weight

Weight course in the studied groups is shown in Figure 1. The animals in DIO groups (HFD and S+HFD) gained more weight than control groups (Figures 1a and b). This was linked with raised initial body weight, final body weight, the percentage of body fat, and Lee index in both sexes in high-fat diet (HFD) groups (Table 1). When Lee index, final body weight, and percentage of body fat were compared between sexes, male rats showed higher body weight, Lee index, and percentage of body fat compared with female rats at the end of the study (Table 1). However, when the percentage of weight changes in HFD and normal diet (ND) female groups were compared, the differences were higher than those of male groups (21% vs. 12%). Also, percentage weight changes were higher than in the F+SHFD group compared with the F+SND group that male groups (24% vs. 13%) (Table 1).

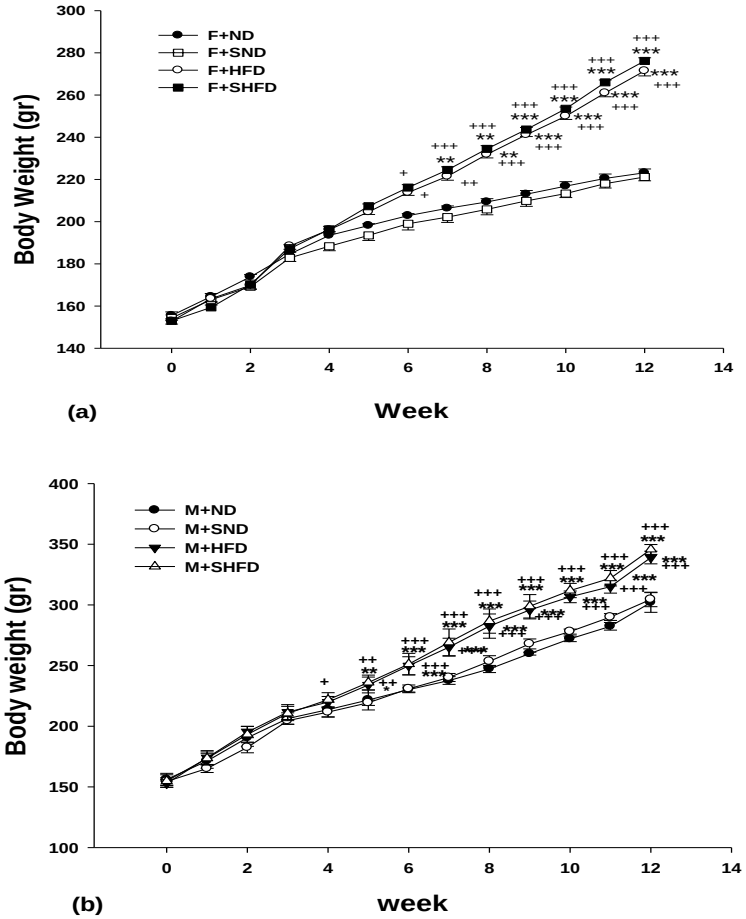

Figure 1. Weight courses in a) female and b) male groups based on the designed regime. Values are expressed as mean±SEM. F+ND: female normal diet group, M+ND: male normal diet group, F+SND: female OVA-sensitized with a normal diet, M+SND: male OVA-sensitized with a normal diet, F+HFD: female high-fat diet group, M+HFD: male high-fat diet group, F+SHFD: female OVA-sensitized with a high-fat diet, F+SHFD: male OVA-sensitized with a high-fat diet. In both sexes, differences between the results of the normal diet group with those of other groups; *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. Differences between sensitized normal diet with obese groups; +: $p<0.05$, ++: $p<0.01$, +++: $p<0.001$. For each group, n=6.

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Table 1. Weight changes and obesity indexes in female and male groups based on the designed regime

variables	ND	S+ND	HFD	S+HFD
Initial body weight (g)				
F	155.5±1.74	154.33±108	152.83±1.37	153±0.96
M	156.16±2.05	154.5±1.23	152.83±1.30	154.83±2.1
p-value	ns	ns	ns	ns
Final body weight (g)				
F	221.16±1.81	223.16±1.81	276.16±1.49	271.5±1.02
M	302±3.32	304.5±2.43	339±2.12	345.67±4.08
p-value	***	***	***	***
Percentage of body weight change (%)				
F	43.30±0.84	43.61±2.24	80.52±1.38	77.71±1.85
M	93.45±2.02	97.12±1.70	121.84±1.19	123.44±3.12
p-value	***	***	***	***
Lee index (mg/mm)				
F	287.31±1.39	286.33±0.83	309.37±1.36	304.97±1.23
M	300.62±0.96	302.62±1.56	316.96±1.85	313.77±0.8
p-value	***	***	**	***
Percentage of body fat (%)				
F	4.74±1.01	4.03±0.60	20.85±0.99	17.64±0.89
M	14.46±0.7	15.92±1.140	26.39±1.35	24.06±0.58
p-value	***	***	**	***

Values are represented as mean±SEM. The t-test was utilized for the analysis of the continuous variable in two sex groups. F: female, M: male, ND: normal diet group, S+ND: OVA-sensitized with a normal diet, HFD: high-fat diet group, S+HFD: OVA-sensitized with a high-fat diet. Differences between gender; ns: no significant, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. For each group, n=6.

Tracheal Response to Methacholine

Figure 2A shows that in female rats, in methacholine concentration-response curves there were leftward shifts in Groups F+SHFD and F+SND compared to Groups F+ND and F+HFD. Moreover, Figure 2B shows that, in male rats, the concentration-response curves to methacholine were shifted to leftward in Groups M+SHFD and M+SND compared to Groups M+ND and M+HFD. Interestingly, DIO caused in OVA-sensitized groups, a leftward shift was considerably evident in M+SHFD and F+SHFD groups.

The EC50 was significantly lower in the tracheal segments of F+SHFD animals than F+ND ($p<0.001$) and F+HFD ($p<0.01$) ones (Figure 2C). In females, the EC50 in the tracheal segments of F+SND was significantly lower than in F+ND ($p<0.01$) and F+HFD ($p<0.05$) groups (Figure 2C), but there was no significant difference with that of F+SHFD. In addition, there was no significant difference in EC50 between F+ND and F+HFD female animals.

In males, the EC50 in tracheal segments of M+SHFD animals was significantly lower than that in M+ND and M+HFD ($p<0.01$, for both) animals (Figure 2D). Furthermore, the EC50 was significantly lower in the tracheal segments of M+SND (0.78±0.04) than M+ND ($p<0.01$) and M+HFD ($p<0.05$) groups (Figure 2D), but there was no significant difference with that of M+SHFD. In addition, there was no significant difference in EC50 between M+ND and M+HFD male animals.

Based on the contractility results, gr force/mg tissue weight (gF/TW) was significantly higher in both OVA-sensitized female groups (F+SND and F+SHFD) than the F+ND group ($p<0.001$, Figure 2E). Also, the gF/TW was significantly higher in F+SHFD rats than the F+HFD group ($p<0.01$, Figure 2E). Although gF/TW was higher in F+HFD animals than the F+ND group, the difference was not significant ($p=0.056$).

Moreover, in M+SND male rats, the gF/TW was significantly higher than that of M+ND and M+HFD groups ($p<0.01$ to $p<0.05$, respectively). In addition, the gF/TW was significantly higher in M+SHFD male animals than M+ND and M+HFD groups ($p<0.001$ to $p<0.01$, respectively). There was no significant difference in gF/TW between M+ND and M+HFD or M+SND and M+SHFD groups (Figure 2F).

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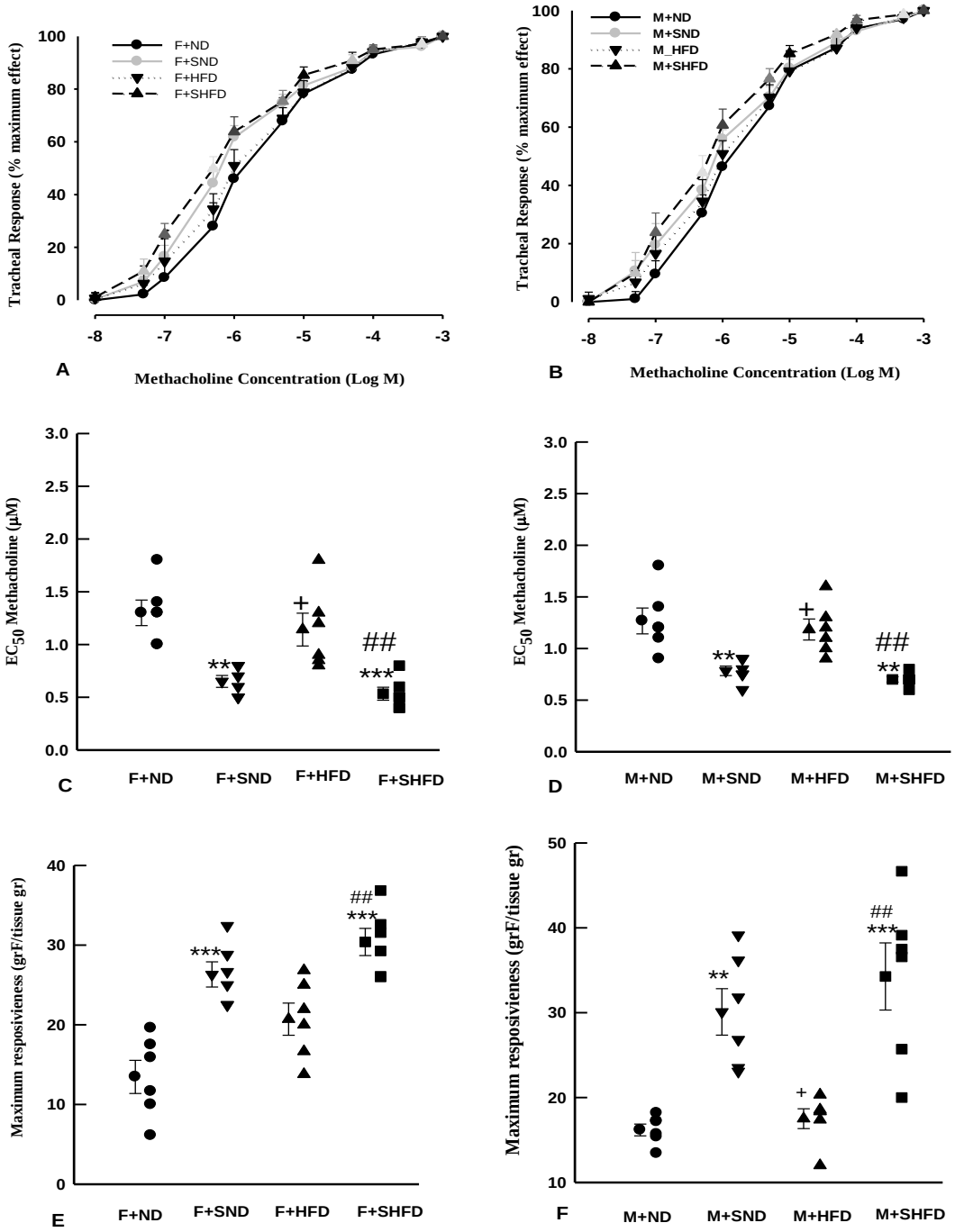

Figure 2. Cumulative log concentration-response curves of methacholine-induced contraction of the isolated trachea in A) female and B) male Wistar rats. Also, individual values and mean+SEM of C) tracheal response to methacholine (EC_{50}, the effective concentration of methacholine, causing 50% of maximum response) in female and D) EC_{50} in male Wistar rats. In addition, tracheal contractility to methacholine (gr force/ tissue gr) (contractility response to 1 mM methacholine) in E) female and F) male rats. F+ND: female normal diet group, M+ND: male normal diet group, F+SND: female OVA-sensitized with a normal diet, M+SND: male OVA-sensitized with a normal diet, F+HFD: female high-fat diet group, M+HFD: male high-fat diet group, F+SHFD: female OVA-sensitized with a high-fat diet, F+SHFD: male OVA-sensitized with a high-fat diet. For each group, n=6. In both sexes, statistical differences between the normal diet and other groups; **: $p<0.01$, ***: $p<0.001$. Statistical differences between OVA-sensitized normal diet with obese groups in both sexes; +: $p<0.05$. Statistical difference between high-fat diet vs. OVA-sensitized high fat diet in both sexes: ##: $p<0.01$.

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Table 2. Parameters of the study in female and male groups based on the designed regime

variables	ND	S+ND	HFD	S+HFD
EC_{50} (µM)				
F	1.3±0.12	0.65±0.05	1.14±0.15	0.53±0.06
M	1.26±0.12	0.78±0.04	1.18±0.10	0.7±0.02
p-value	ns	ns	ns	ns
Maximum Response (grF/tissue gr)				
F	13.46±2.07	26.31±1.57	20.70±2.01	30.39±1.70
M	16.18±0.69	30.08±2.73	17.51±1.16	34.26±3.95
p-value	ns	ns	ns	ns
Insulin (MIU/mL)				
F	2.05±0.04	3.16±0.08	3.28±0.12	4.05±0.15
M	2.15±0.04	2.8±0.06	3.83±0.26	3.25±0.07
p-value	ns	**	ns	**
HOMA-IR				
F	0.38±0.01	0.74±0.02	0.76±0.02	0.98±0.04
M	0.41±0.01	0.61±0.01	0.96±0.07	0.68±0.01
p-value	ns	**	*	***
QUICK				
F	0.45.1±0.002	0.40±0.002	0.39±0.003	0.38±0.003
M	0.44±0.003	0.41±0.001	0.38±0.005	0.40±0.005
p-value	ns	**	*	**
FBS (mg/dl)				
F	76.33±1.56	94.50±1.14	94.66±1.02	98.16±2.22
M	78.33±1.05	89.16±1.64	101.5±2.51	86±0.85
p-value	ns	*	*	**
BS-30 (mg/dl)				
F	100.33±5.74	115.83±1.58	113.17±2.40	132.5±4.01
M	126.50±3.07	127.33±2.47	130.67±2.97	110.17±2.15
p-value	**	**	**	**
BS-60 (mg/dl)				
F	88.83±5.33	105.50±2.5	104.17±3.76	124±4.86
M	104.17±2.94	112.83±3.19	124.83±2.52	107.67±2.1
p-value	*	ns	**	*
BS-90 (mg/dl)				
F	88.50±1.54	102.50±4.51	95.66±2.34	111±3.13
M	90.66±2.59	94.66±1.94	117.17±4.88	99.66±2.26
p-value	ns	ns	**	*
BS-120 (mg/dl)				
F	76.16±3.70	100.50±2.92	90±2.9	105±5.76
M	85±2.69	91.50±1.87	109.83±4.2	97.50±2.24
p-value	ns	*	**	ns

Values are represented as mean±SEM. F: female, M: male, ND: normal diet group, S+ND: OVA-sensitized with a normal diet, HFD: high-fat diet group, S+HFD: OVA-sensitized with a high-fat diet, EC_{50}: effective concentration of methacholine, causing 50% of maximum response, HOMA-IR: insulin resistance estimation using homeostasis model assessment method, QUICK: quantitative insulin sensitivity check index, FBS: first blood sugar, BS: blood sugar in different times after oral glucose tolerance test. Differences between gender; ns: no significant, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. For each group, n=6.

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Effect of Sex on Tracheal Response to Methacholine

When the EC_{50} was compared between sexes, F+SHFD rats showed a statistically low EC_{50} compared with the M+SHFD group ($p<0.05$). On the other hand, although the EC_{50} was low in F+SND rats compared to M+SND rats, the difference was not statistically significant (Table 2).

The gF/TW sex differences showed that there were no differences between groups (Table 2).

Blood Glucose Levels

Figure 3a and 3b indicate blood glucose levels before and after an oral glucose tolerance test (OGTT) in female and male rats. The plasma glucose level in all groups increased to the peak level 30 min after glucose administration. The blood glucose levels were summarized in Table 2.

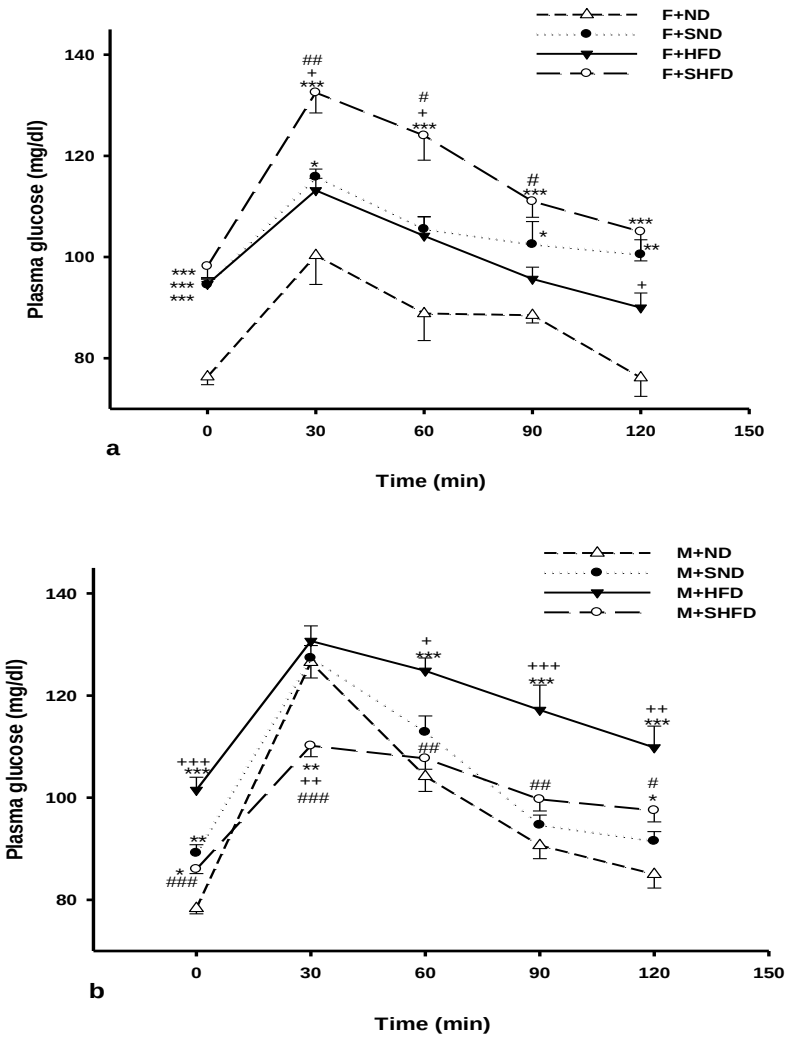

Figure 3. Plasma glucose levels after an oral glucose tolerance test in a) female and b) male Wistar rats based on the designed regime. F+ND: female normal diet group, M+ND: male normal diet group, F+SND: female OVA-sensitized with a normal diet, M+SND: male OVA-sensitized with a normal diet, F+HFD: female high-fat diet group, M+HFD: male high-fat diet group, F+SHFD: female OVA-sensitized with a high-fat diet, F+SHFD: male OVA-sensitized with a high-fat diet. For each group, n=6. In both sexes, statistical differences between the normal diet and other groups; *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. Statistical differences between OVA-sensitized normal diet with obese groups in both sexes; +: $p<0.05$, ++: $p<0.01$, +++: $p<0.001$. Statistical difference between high-fat diet vs. OVA-sensitized high fat-diet in both sexes: #: $p<0.05$, ##: $p<.0.01$, ###: $p<0.001$.

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Serum Insulin Levels, Insulin Resistance Index (HOMA-IR), and Insulin Sensitivity Index (QUICKI)

Figure 4A and B show fasting/basal serum insulin levels in female and male experimental groups. In female experimental groups, results demonstrated that serum insulin levels were lower in F+ND than other groups (p<0.001). On the other hand, serum insulin levels were higher in F+SHFD than F+SND and F+HFD groups (p<0.001 for both) (Figure 4A).

However, in male experimental groups, serum insulin levels were lower in M+ND group than M+ND (p<0.05), M+HFD (p<0.001), and M+SHFD groups (p<0.001). In addition, serum insulin levels were higher in M+HFD animals than M+SND (p<0.001) and M+SHFD groups (p<0.05) (Figure 4B).

The HOMA-IR differences in female experimental groups showed that F+ND rats had the lowest HOMA-IR when compared with other groups (p<0.001). Additionally, the amount of HOMA-IR was higher in F+SHFD than F+SND and F+HFD groups (p<0.001 for both) (Figure 4C).

Nevertheless, in male experimental groups, HOMA-IR was lower in M+ND when compared with M+SND (p<0.05), M+HFD (p<0.001), and M+SHFD groups (p<0.01). Also, HOMA-IR was higher in M+HFD than M+SND and M+SHFD groups (p<0.001 for both) (Figure 4D).

The QUICKI differences in female experimental groups indicated that F+ND rats had the highest amount OUICKI when compared with other groups (p<0.001). Additionally, the amount of QUICKI was lower in F+SHFD than F+SND (p<0.001) and F+HFD groups (p<0.01) (Figure 4E).

In male experimental groups, however, QUICKI was lower in M+HFD when compared with M+ND (p<0.001), M+SND (p<0.001), and M+SHFD groups (p<0.01). Also, the QUICKI was higher in M+ND in comparison with M+SND and M+SHFD groups (p<0.001 for both), and there were no significant differences between M+SND and M+SHFD groups (Figure 4F).

Effect of Sex on Serum Insulin Levels, HOMA-IR, and QUICKI

Insulin serum levels were higher in F+SND than M+SND group (p<0.01). In addition, serum insulin levels were higher in F+SHFD than M+SHFD (p<0.01) (Table 2).

The amount of HOMA-IR was higher in F+SND than M+SND (p<0.01). Moreover, HOMA-IR was higher in M+HFD than F+HFD (p<0.05). Additionally, HOMA-IR was higher in the F+SHFD group than the M+SHFD group (p<0.001) (Table 2).

The amount of QUICKI was lower in F+SND rats than M+SND animals (p<0.01). On the other hand, QUICK was higher in M+SHFD than F+SHFD (p<0.01) (Table 2). The analysis of QUICKI in HFD groups in both sexes revealed that QUICKI was significantly higher in F+HFD than M+HFD (p<0.05) (Table 2).

Correlation Analysis of Variables of Study with EC50

Table 3 presents the correlation between some parameters of study and EC50. Results showed that, in female groups, there was negative correlation between serum insulin (r=-0.654, p<0.001), FBS (r=-0.512, p<0.011), and HOMA-IR (r=-0.637, p<0.001), and a positive correlation between EC50 and QUICKI (r=0.632, p<0.001). Nevertheless, there was no significant correlation between EC50 and the variables of study in male groups.

Table 3. Pearson correlation analysis of the variables of study and EC50 (the effective concentration of methacholine, causing 50% of maximum response) levels

Variables	EC50			
	Female		Male	
	r	*P-value*	*r*	*p-value*
Percentage of body weight changes (%)	-0.109	0.612	-0.152	0.477
Lee index (mg/mm)	-0.041	0.848	-0.108	0.617
Percentage of body fat (%)	-0.041	0.848	-0.108	0.617
Insulin (MIU/mL)	-0.654	0.001	-0.191	0.373
Fasting blood sugar (mg/dl)	-0.512	0.011	-0.007	0.973
Homeostatic model assessment (HOMA-IR)	-0.637	0.001	-0.095	0.657
Quantitative insulin sensitivity check index (QUICKI)	0.632	0.001	0.221	0.300

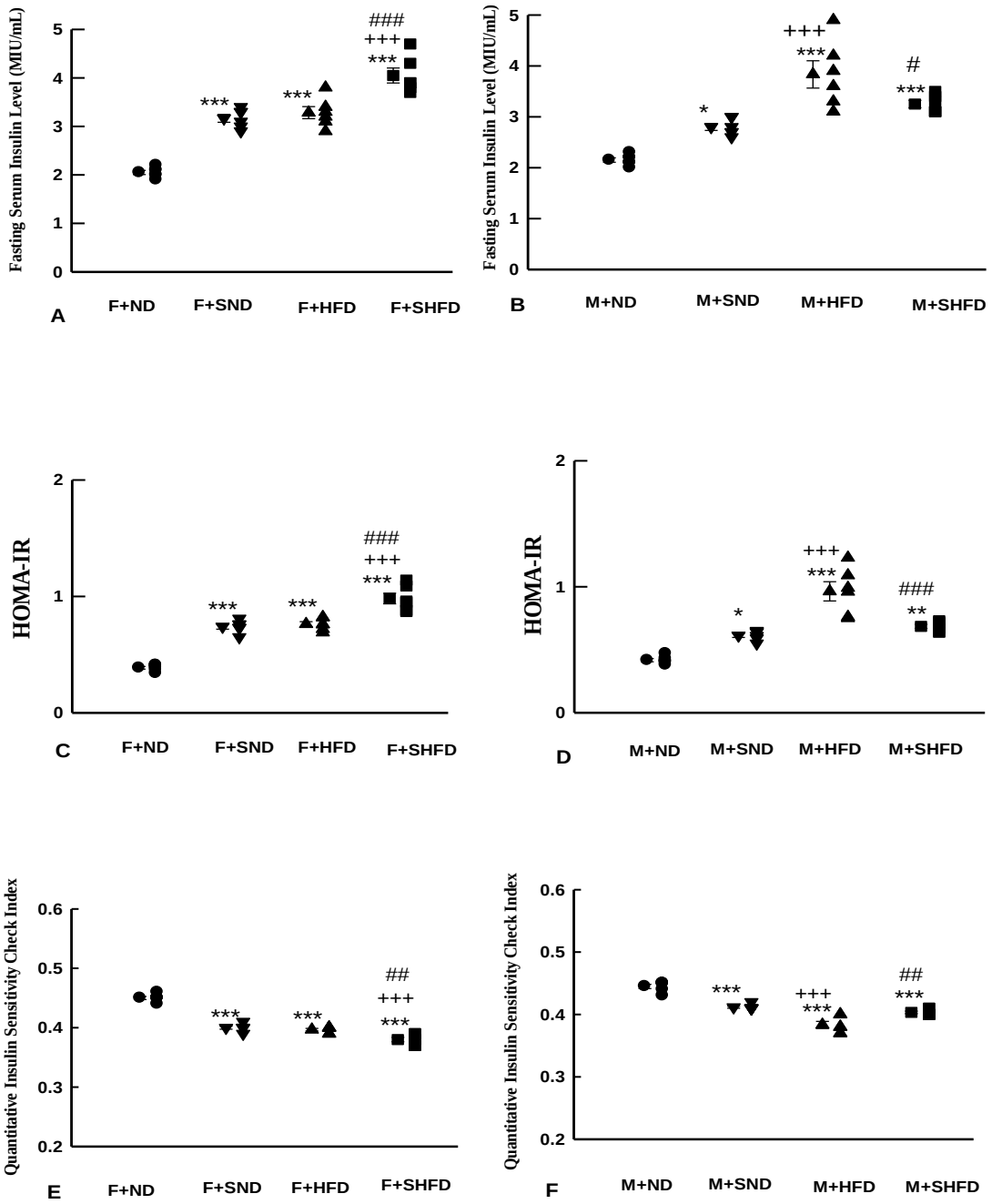

Figure 4. Individual values and mean±SEM of fasting/basal serum insulin levels (A: female, B: male), insulin resistance index, HOMA-IR (homeostasis model assessment) (C: female, D: male), and Quantitative insulin sensitivity check index (QUICKI) (E: female, F: male) in experimental groups. F+ND: female normal diet group, M+ND: male normal diet group, F+SND: female OVA-sensitized with a normal diet, M+SND: male OVA-sensitized with a normal diet, F+HFD: female high-fat diet group, M+HFD: male high-fat diet group, F+SHFD: female OVA-sensitized with a high-fat diet, F+SHFD: male OVA-sensitized with a high-fat diet.. For each group, n=6. In both sexes, statistical differences between the normal diet and other groups; *: $p<0.05$, ***: $p<0.001$. Statistical differences between OVA-sensitized normal diet with obese groups in both sexes; +++: p<0.001. Statistical difference between high-fat diet vs. OVA-sensitized high-fat diet in both sexes: #: $p<0.05$, ###: $p<.0.001$.

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DISCUSSION

The present study indicated that, in both sexes, tracheal responsiveness to methacholine increased in OVA-sensitized groups; with a considerable increase in obese rats. In addition, AHR was higher in female obese OVA-sensitized rats comparison to male groups. Also, insulin concentration and HOMA-IR were higher and QUICK index was lower in female obese OVA-sensitized rats comparison to male groups. Furthermore, results revealed an association between AHR and increased insulin level and HOMA-IR only in the female sex.

In this study, the model used to induce obesity was based on a high-fat diet. The results indicated that DIO results in elevated body weight and obesity indexes in obese groups, consistent with the finding of previous studies.[22,25] Moreover, some human asthma characteristics are BALF eosinophilia, AHR, and smooth muscle hypertrophy. OVA-sensitization in animals showed some aspects of human asthma, including AHR and increased maximum response, which were similar to another study.[29]

Results indicated that airway responsiveness to methacholine in both sexes was increased in sensitized obese and lean rats compared with wild-type controls. This finding confirmed the previous study in AHR development in obese mice. The novelty of our study compared with previous studies in the assessment of AHR was that sexual dimorphism was evident in obesity associated with OVA-sensitization statue. Previous studies generally focused on the airway responsiveness in obesity associated with asthma independent of sex.[30] Our results showed that a high-fat diet in female OVA-sensitized rats was very susceptible to AHR compared with male rats. Indeed, it is clear from the results that obesity in OVA-sensitized condition has the most impact on the tracheal responsiveness of females.

In animal studies of asthma associated with obesity, it has been reported that AHR is a common feature in obese animals.[31] In our study, in both sexes, although the EC_{50} curve shifted to the left in HFD groups when compared with ND groups, the shift was not statistically significant. This difference can be due to either the direct assessment of the effect of AHR on trachea or the sample size used for this study. In both sexes in the HFD group, the shift of the EC_{50} curve, at least in part, shows the lung inflammation caused by the localized release of adipocytokines in lung tissue or systemic inflammation. We have previously shown that visfatin, one of the adipocytokines, in lung tissue increases in obesity associated with the OVA-sensitization condition.[32] The accumulation of inflammatory cytokines along with systemic inflammation in obesity situation may have affected airway responsiveness, which requires further studies.

The present study also revealed sex differences in the AHR assessing to the methacholine, which was more pronounced in the female obese OVA-sensitized animal. In fact, sensitization with OVA caused AHR in both sexes, but after diet-induced obesity and OVA-sensitization, the AHR in the female group was greater than that of the male group. A number of studies have evaluated the association between sex hormones and asthma. In human studies, a sex-related difference in risk, incidence, and pathogenesis of pulmonary disease has been identified.[19] Additionally, animal studies have shown that female rats are more susceptible to allergic airway diseases than male rats.[19] Several mechanisms have been proposed for sex dimorphism in allergic airway disease, such as increased serum immunoglobulin E (IgE) in allergic female mice,[33] increased bronchial-bronchiolar inflammation in female mice,[34] the role of estrogen in increased airway inflammation in female mice,[35] reduction in Treg cells in female mice,[36] and protective role of androgen in the development of allergic airway disease.[34] In our study, due to the increased AHR in the obese OVA-sensitized female group compared with the male group, it can be concluded that a number of factors mentioned above have a causal role which requires further investigation.

In an effort to further understanding of the sex differences in obesity associated with OVA-sensitization, glucose level, insulin level, and insulin resistance indexes were tested in study groups. Indeed, it has been shown that obesity may have an effect on airway inflammation in the obese individual with asthmatic.[37] It has been reported that obesity may cause the metabolic complications such as impaired glucose uptake, diabetes mellitus, insulin resistance, and cardiovascular diseases.[38] Meanwhile, insulin resistance plays an important role in metabolic complications associated with obesity.[38] Several clinical and epidemiological studies have reported that decreased lung function was associated with elevated serum glucose level and insulin resistance.[39,40] However, although AHR is the main characteristic of

asthma, little information is available on the association between AHR and insulin resistance. The results of the present study showed that using a combination of high-fat diet and OVA-sensitization causes increased insulin concentration as well as increased HOMA-IR in both sexes. Interestingly, this study showed that a sex difference exists in insulin resistance and insulin level in obesity and OVA-sensitization. In the obese male group (M+HFD), we observed increased insulin and HOMA-IR levels compared with the female obese group, which was in line with another study.[41] In addition, it was shown in another study that insulin resistance is higher among patients with asthma, in line with our results.[42] However, sensitization with OVA after diet-induced obesity caused increased insulin and HOMA-IR levels in the female group when compared with the male group. Indeed, insulin resistance was higher in female obese OVA-sensitized rats than male ones. Also, we found that there is only a negative correlation between insulin resistance and AHR in the female obese OVA-sensitized group.

Previous studies have shown that insulin resistance and metabolic imbalances can affect pulmonary function through several mechanisms. It is known that the insulin growth factor-1 (IGF-1) is able to modify the smooth muscle contractility and promote its proliferation.[43] Adipocytokines such as leptin, adiponectin, resistin, and visfatin also affect the severity of asthma or pulmonary function.[44] Adipocytokines, with pro-inflammatory properties, cause to the recruitment of inflammatory cells such as neutrophils into the lungs.[18] Animal studies have indicated that adipocytokines and cytokines such as interluekine-6 (IL-6), interluekine-1beta (IL-1β), and tumor necrosis factor alpha (TNF-α), cause airway inflammation and worsen asthma conditions.[18] Based on previous studies, there is a relationship between insulin resistance in healthy humans and AHR.[45] Insulin resistance has also been identified as a result of allergic airway inflammation in the mouse model of asthma.[46] In the present study, it was found that OVA-sensitization leads to insulin resistance in obese and lean groups in both sexes. However, the increase in insulin resistance (or decreased insulin sensitivity) in OVA-sensitization situation was more pronounced in females than male. Indeed, in our study, insulin resistance was a significant risk factor for AHR in male and female rats, especially for females. In a human study, a strong link has been reported between metabolic syndrome and asthma in women more than men.[18]

Several mechanisms have been proposed for increased AHR as a result of insulin resistance although the exact mechanism is not clear. Recently, animal studies have shown that insulin is able to induce hypercontractility in airway smooth muscle.[18] In patients with diabetes, inhaled insulin has been shown to lead to a reduction in pulmonary function through airway smooth muscle contraction.[16] Therefore, the characteristic of the metabolic syndrome is insulin resistance, possibly leading to hyperinsulinemia and affecting airway smooth muscle mass or contraction. On the other hand, it has been reported that hyperinsulinemia, hyperglycemia, and increased IGF-1 may contribute to hypersecretion of mucus as well as AHR.[47] Another effect of increased insulin/IGF-1 can be the proliferation and differentiation of fibroblasts, which leads to collagen deposition and fibrosis.[48] Metabolic syndrome may lead to the remodeling of the airway (even in the absence of allergy condition) and reduce pulmonary function.

In summary, insulin resistance and metabolic syndrome are associated with AHR in obese OVA-sensitized male and female rats. Interestingly, AHR was more evident in female obese OVA-challenged rats. Thus, metabolic syndrome and insulin resistance may contribute to the pathogenesis of obese OVA-sensitized rats and sex dimorphism.

ACKNOWLEDGEMENTS

This study was funded by Ardabil University of Medical Sciences. The authors would like to thank the physiology department of Tabriz University of Medical Sciences for supporting this study.

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