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Research Article

ENDOCRINOLOGICAL ROLE OF LEPTIN IN OBESITY AND ASTHMA

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ABSTRACT

Objective: Role of leptin resistance in correlation between obesity and asthma.

Methods: High-caloric diet was given for 8 weeks to induce obesity. Ovalbumin followed by aluminum hydroxide was given to induce asthma. The animal was treated with leptin analog (0.4 mg/kg, i.p. for 7 days) and leptin antagonist (3 mg/kg, p.o., for 7 days). Biochemical parameters such as serum leptin, ghrelin, and tumor necrosis factor alpha (TNF-α) and physical parameters such as tidal volume and airflow rate were estimated to confirm the state of asthma and obesity, respectively.

Results: It was found that leptin and ghrelin were elevated in obese and obese asthmatic condition, responsible for leptin resistance. Treatment with leptin analog and leptin antagonist significantly increases and decreases serum leptin levels, respectively. There was no significant change in TNF-α and ghrelin level after leptin analog treatment. The result of respiratory parameters improved with leptin analog. From our study, we found beneficial role of leptin analog in obese asthmatic condition.

Conclusion: Leptin is an alternative treatment approach to treat obese asthmatic condition.

Keywords: Obesity, Asthma, Leptin, Leptin antagonist, Tumor necrosis factor alpha.

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INTRODUCTION

Obesity is one of the inflammatory conditions characterized by fat deposition in the adipose tissue [1,2]. Obesity is linked with several disorders including cardiovascular diseases, certain types of cancer [3], and type-2 diabetes and it also raises the risk of pulmonary problems including asthma [4,5]. It is a strong risk factor for mortality in adulthood. The study revealed that overall 20% of males and 30% of females are obese worldwide [4]. The reason is due to rapid urbanization and westernization of countries. This leads to consumption of larger amounts of food with decreased physical activities, results in higher chances of obesity [5].

Asthma is chronic inflammatory respiratory disorder characterized by airway hyperresponsiveness that leads to recurrent episodes and reversible airflow obstruction [6]. Obesity has been significantly associated with asthma in both genders [7]. Studies indicated that obesity may increase asthma severity and reduce the efficacy of standard asthma medications [8]. Various clinical cohort studies showed that over 300,000 adults whose body mass index >25 were diagnosed with asthma [9].

It is well established that insulin, leptin, and ghrelin levels are significantly altered in obesity and asthma [10]. Leptin and ghrelin regulate satiety center by acting on brain centers. Both control the food intake through acting on neuropeptide Y pathway. Various studies showed that obesity is due to either leptin resistance or elevated serum level [3-5]. Clinical studies also revealed higher level of leptin in asthmatic patients [11].

Both asthma and obesity are chronic inflammatory conditions [9]. Leptin is an inflammatory hormone secreted from adipose tissue [12]. The obese state could lead to asthma in those without prior airway disease in a number of different ways. These include changes in lung structure and function related to diet and mechanics, oxidative stress signaling, cytokine derangement, and neuronal signaling pathways. Growing evidence also suggests that the pro-inflammatory effects of leptin may contribute to the higher incidence of asthma in the obese population [13,14].

At present, there are limited treatment options available for these two comorbid conditions. Even current treatments also have numerous side effects. Therefore, there is a need for identification of newer cost-effective treatment approach for asthma with obesity. The present investigation was done to find possible role of leptin in pathogenesis of asthma with obesity. We investigated role of leptin through high-caloric diet-induced obesity in ovalbumin (OVA)-induced asthma in Swiss albino mice.

METHODS

Swiss albino mice of female sex weighing between 24±6 g were obtained from the central animal house of Faculty of Pharmacy, Dharmsinh Desai University, Nadiad. The animal studies were approved by the Institutional Ethics Committee (FOP/06/17), ratified by the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India. Animals were naive to drug treatment and experimentation at the beginning of all studies. Animals were kept individually in polypropylene cages in an environmentally controlled room of the animal house and maintained at a temperature of 25±2°C with a 12 h dark and light cycle. 10 days of acclimatization were provided to animal. The animals were provided water and food *ad libitum*. Mice were fed with laboratory pellet chow diet or special high-caloric diet according to the protocol. Composition of experimental diet (gm/kg diet) was according to Soni *et al.* [7].

Experimental design
A total of 60 mice were used and divided into 10 groups (n=6).

Group I: (Normal control mice) Animals were maintained on laboratory pellet chow diet and water *ad libitum* for 12 weeks. No treatment was given to these mice.

Group II: (Obese control mice) Animals were maintained on the high-caloric diet for 8 weeks to induce obesity.

Group III: (Asthma control mice) Animals were maintained on laboratory pellet chow diet for up to 8 weeks, and then, induction phase of asthma was started. Mice were sensitized with OVA conjugated to aluminum hydroxide and challenged with saline to induce asthma. The induction with OVA was done on day 1–day 23 and challenge was given for every 7th day for 3 weeks.

Group IV: (Obese asthmatic control mice) Animals were given with high-caloric diet control mice maintained for 8 weeks, and then, induction phase of asthma was started. Mice were sensitized with OVA conjugated to aluminum hydroxide and challenged with saline to induce asthma. The induction with OVA was done on day 1–day 23 and challenge was for every 7th day for 3 weeks.

Group V: (Leptin analog-treated obese mice) Animals were fed with high-caloric diet as mentioned in Group-II and these animals were treated with leptin analog (0.4 mg/kg, i.p. for 7 days) during the 9th week of study.

Group VI: (Leptin analog-treated asthmatic mice) Animals were treated same as mentioned in Group-III and these animals were treated with leptin analog (0.4 mg/kg, i.p. for 7 days) during the 9th week of study.

Group VII: (Leptin analog-treated obese asthmatic mice) Animals were treated same as mentioned in Group-IV and these animals were treated with leptin analog (0.4 mg/kg, i. p. for 7 days) during the 12th week of study.

Group VIII: (Leptin antagonist-treated obese mice) Animals were fed with high-caloric diet as mentioned in Group-II and these animals were treated with leptin antagonist (3 mg/kg, p. o., for 7 days) during the 9th week of study.

Group IX: (Leptin antagonist-treated asthmatic mice) Animals were treated same as mentioned in Group-III and these animals were treated with leptin antagonist (3 mg/kg, p. o., for 7 days) during the 9th week of study.

Group X: (Leptin antagonist-treated obese asthmatic mice) Animals were treated same as mentioned in Group-IV and these animals were treated with leptin antagonist (3 mg/kg, p. o., for 7 days) during the 12th week of study.

At the end of experimental period, the animals were anesthetized with ketamine, following overnight fasting. Blood was drawn by retro-orbital method. Serum was separated by centrifugation at 4000 rpm (revolution per minute). Serum levels of leptin, ghrelin, and tumor necrosis factor-α (TNF-α) were measured using standard ELISA kits. The serum samples were stored at −70°C until analysis.

Measurement of respiratory parameters
Tidal volume, respiratory rate, and airflow rate were measured for the assessment of asthmatic condition. Tidal volume is the volume of air inhaled or exhaled per breath during normal breath. The measurement of tidal volume was done with the help of respiratory volume transducer using student physiograph. The physiograph was calibrated with the help of 0.02 cc and 0.1 cc volume air supplier. The tidal volume was measured in terms of height of the response obtained. Then, the height was converted to volume [15].

Respiratory rate is the number of breath per unit time. The respiratory waves recorded for the tidal volume was counted for a period of 1 min and expressed as breaths per minute. Airflow rate is a volume of air inspired or expired per unit time during normal breathing [15]. It was calculated as follows:

Airflow rate=Respiratory rate*tidal volume.

Chemicals and diagnostic kits
Leptin analog (recombinant Mouse leptin - cyt-31), leptin antagonist (MBS 400080 Pegylated mouse super leptin antagonist - mutant D23/L39A/D40A/F414A), ELISA kit for leptin (ELM-leptin-1 Mouse leptin ELISA 1*96 well), ghrelin (EIAM-GHR-1 Mouse EIA 1*96 well), and TNF-α (ELM-TNF-α-1 mouse TNF-α ELISA 1*96 well) were purchased from Everon Life Sciences, New Delhi, India.

Statistical analysis
Statistical evaluation of analytical data was done by one-way analysis of variance followed by Tukey's test using statistical software GraphPad Prism 3.0. Data were expressed as mean ± standard error of the mean and statistically significant was determined at $p<0.05$.

RESULTS

Endocrinological effect of leptin and leptin antagonist in the state of obese asthmatic condition was evaluated using various physical and biochemical parameters. The results of physical parameters are given in Table 1 while biochemical parameters represented by graphical presentation.

Effect of leptin analog and antagonist on body weight
Obesity was induced in normal mice by feeding hypercaloric diet for 8 weeks. The mean body weight of the disease control animals was compared to the control animal. The mean body weight of leptin analog-treated obese, asthmatic, and obese asthmatic group was compared to the obese, asthmatic, and obese asthmatic animal, respectively. Same was done for leptin antagonist-treated animals.

There was a significant (Group I, *$p<0.05$) increase in calorie intake per week among the high-caloric diet mice as compared to normal diet-fed mice while in case of treatment with leptin and leptin antagonist, changes in caloric intake were observed when compared to standard disease control group (Table 1). There was significant decrease in caloric intake after leptin treatment when compared to disease control animal (Group II, III, and IV as @, #, and $p<0.05).

Effect of leptin analog and antagonist on respiratory parameters
The tidal volume and airflow rate were measured as respiratory parameters. It was observed that tidal volume and airflow rate of obese, asthma, and obese asthmatic animals were significantly decreased when compared to control animal (Group I, *$p<0.05$) and significantly increased when compared to disease control animal (Group II, III, and IV as @, #, and $ $p<0.05$) with leptin treatment. It was also observed that both parameters were worsening in treatment with leptin antagonist (Fig. 1).

Effect of leptin analog and antagonist on biochemical parameters
7 days administration of leptin analog in disease control groups revealed significant decrease in serum leptin level in obese and obese asthmatic group when compared to control (Group I, *$p<0.05$). It was observed that significantly increase in leptin-treated animals when compared to disease control animal (Group II, III, and IV as @, #, and $p<0.05$) while significant decrease in leptin antagonist-treated animals when compared to disease control group.

Administration of leptin analog in disease control groups revealed significant increase in serum TNF-α level in obese and obese asthmatic group when compared to control (Group I, *$p<0.05$). It was observed that significantly decrease in leptin-treated animals when compared to disease control animal (Group II, III, and IV as @, #, and $p<0.05$) while

Table 1: Effect of leptin analog and antagonist on body weight

Groups (weeks)	I	II	III	IV	V	VI	VII	VIII	IX	X
1	22±0.32	20±0.14	24±0.34	21±0.34	23±0.64	21±0.14	23±0.21	24±0.36	22±0.09	23±0.64
2	24±0.54	26±0.29	24±0.20	22±0.28	24±0.36	24±0.38	23±0.15	28±0.26	24±0.20	22±0.28
3	25±0.53	30±0.16	25±0.15	26±0.36	31±0.26	31±0.21	31±0.11	32±0.17	31±0.29	31±0.21
4	31±0.14	45±0.58	29±0.78	45±0.74	41±1.70	33±0.46	43±0.43	37±0.86	28±0.46	42±0.43
5	34±0.28	54±0.82	32±0.17	53±1.29	44±2.00	41±0.29	48±0.75	40±0.75	31±0.82	50±0.75
6	36±0.20	53±0.82	38±0.17	53±0.85	48±1.15	40±0.14	55±0.79	41±0.88	31±0.82	46±0.71
7	38±0.13	54±0.79	41±0.11	54±0.99	54±1.05	41±0.09	56±0.72	42±0.74	31±0.66	49±0.79
8	39±0.13	55±0.55	41±0.25	56±0.90	59±0.81	42±0.14	58±0.65	42±0.53	32±0.60	52±0.61
9	38±0.46	56±1.05	42±0.43	59±0.91	61±1.49	41±0.45	59±0.86	47±0.86	39±0.45	57±0.86
10	36±0.48	57±0.86	41±0.43	60±0.46	59±1.15	41±0.45	60±0.91	53±0.43	39±0.45	55±0.68
11	35±0.34	58±0.91	42±0.43	59±0.82	49±2.13	30±0.33	51±1.23	59±0.97	45±0.96	58±0.43
12	35±0.14	59±0.63*	42±0.53	59±0.58*	30±0.68@	18±0.38#	27±0.67$	63±1.01	59±0.35	62±0.34

*p<0.05 when compared with Group-I. @p<0.05 when compared with Group-II. #p<0.05 when compared with Group-III. $p<0.05 when compared with Group-IV

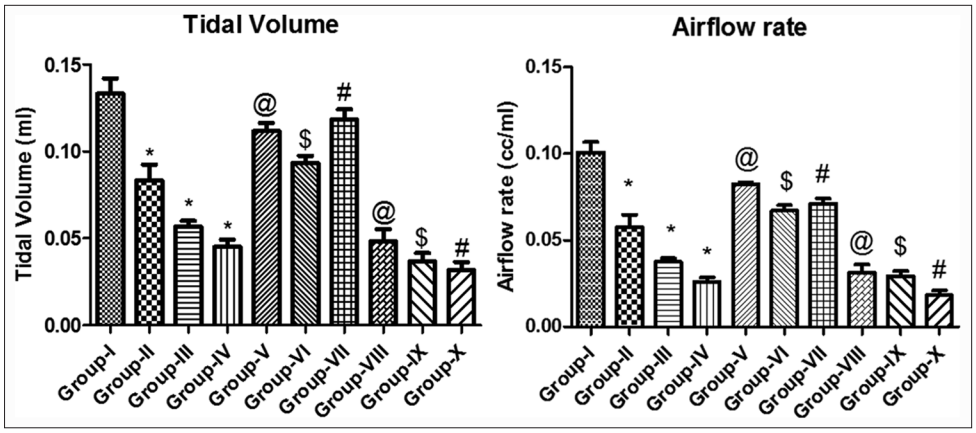

Fig. 1: Effect of leptin analog and leptin antagonist on tidal volume and respiratory flow rate. *p<0.05 when compared with Group-I. @ p<0.05 when compared with Group-II. #p<0.05 when compared with Group-III. $p<0.05 when compared with Group-IV

no changes were observed with leptin antagonist-treated animals. Serum ghrelin level remains unchanged in all groups (Fig. 2).

DISCUSSION

Obesity is a disorder of inflammation and energy imbalance, occurring when calorie expenditure is less compared to high-caloric food intake [16]. Asthma symptoms such as dyspnea and wheezing appear as a result of excess of thoracic and abdominal fat deposition [17]. Despite complex etiological factors for both conditions, leptin resistance found to be one of the causes of asthmatic symptom in obesity [18]. In the present study, we evaluated role of leptin resistance in pathogenesis of asthma in obese condition and also focused on the effect of exogenous leptin and leptin antagonist in this comorbid condition.

Leptin, an adipokine hormone, inhibits food intake and increases energy expenditure by central action on hypothalamus [19,20] while ghrelin reported to be involved in increasing food intake [21]. Leptin effect is, therefore, antagonistic to the ghrelin effect. Previous studies of Rosická *et al.* reported that serum level of leptin and ghrelin was elevated in obesity [22]. This both hormone's actions is due to their role of regulating energy homeostasis through the changes in neuropeptide Y secretion [23]. In our study, it was found that administration leptin analog reduced the body weight of animals while administration of leptin antagonist increased body weight of animals when compared to diseased control group at the 12th week. In same comparison, ghrelin levels were significantly higher. The reason for such elevation would be leptin resistance [18]. Previously, it was found that leptin resistance is one of the factors for pathogenesis of obesity [10] and now suggested to be involved in the development of asthma in obesity. Thus, we proposed that leptin resistance was accompanied by increased in serum ghrelin and serum leptin levels [22,23]. Clinical and preclinical studies suggested that obesity-induced raise in leptin level and leptin resistance would be responsible for worsening asthma symptoms [23].

We investigated changes in serum leptin, TNF-α, and ghrelin level in animals with obesity, asthma, and obese asthmatic condition after exogenous leptin analog and leptin antagonist. Leptin and TNF-α levels were significantly increased in obese animals and obese asthmatic animals when compared with normal control animals. It has been previously reported that leptin increases the level of inflammatory mediators such as TNF-α [23]. Furthermore, respiratory parameters tidal volume and airflow rate were significantly decreased in diseased control animals. Therefore, it may be suggested that leptin resistance aggravates the asthmatic condition.

In the present study, it was observed that leptin administration improve the state of leptin resistance as observed with reduction in body weight at the 12th week. Administration of leptin antagonist significantly decreases serum level of leptin. Hence, decrease in serum leptin level may be responsible for the development of asthma. Furthermore, elevated level of TNF- α in leptin antagonist treated group worsens the symptoms of asthma. It is well established that serum level of TNF-α was significantly reduced to produce anti-inflammatory action by treatment with leptin analogue [24,25]. Respiratory parameter was significantly increased in disease control animal when treated with exogenous leptin while leptin antagonist reduces respiratory parameter. Thus, leptin antagonist ultimately potentiates weight gain (due to blocking of leptin effect) and thereby worsen the state of asthma. Hence, we may conclude that leptin could be alternative treatment for resistant type of asthma.

CONCLUSION

From our study, we conclude that higher level of serum leptin may be involved in the development of leptin resistance and thus induce

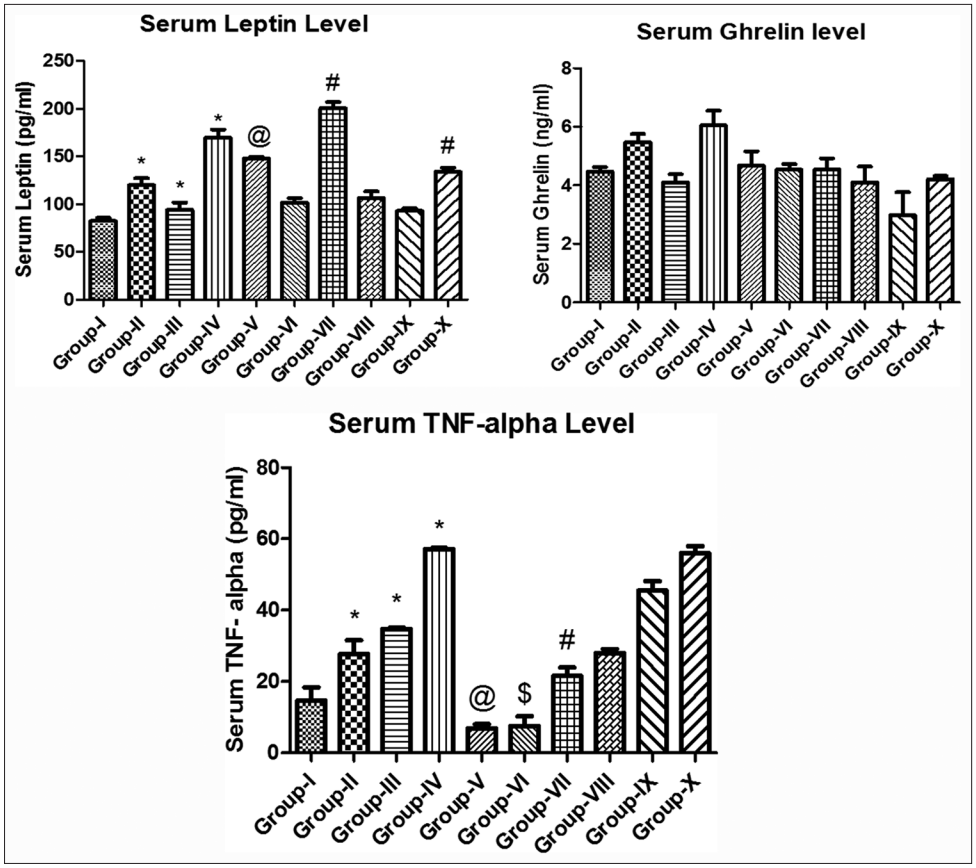

Fig. 2: Effect of leptin analog and leptin antagonist on biochemical parameter such as serum leptin, tumor necrosis factor alpha, and ghrelin levels. *p<0.05 when compared with Group-I. @p<0.05 when compared with Group-II. #p<0.05 when compared with Group-III. $p<0.05 when compared with Group-IV

obesity and asthma. These comorbid conditions could be improved by leptin analog (by improving state of leptin resistance). However, further studies are needed to determine clinical efficacy of leptin analogs in patient of asthma-associated obesity. It is needed the effect of leptin analog with standard drugs of asthma and obesity.

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AUTHORS' CONTRIBUTION

Arun K Soni has provided designs, innovations, and performed the experiment in the laboratory. Anand B Pithadia has minor role in conducting experiment in laboratory, preparation of manuscript, and analysis of obtained data. Shrikalp S Deshpande has provided intellectual content along with mentorship. He is also guarantor for genuinely work done.

Bhanubhai N Suhagia has guided for the preparation of manuscript and protocol for conducting experiment.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding publication of this article.

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