Resistin, adiponectin and leptin levels in overweight patients with asthma

Valores de resistina, adiponectina e leptina em doentes com asma e excesso de peso

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ABSTRACT

Recent years have seen a greater prevalence of asthma and obesity. Asthma is a chronic inflammatory disease of the airways. Obesity is an imbalance between energy intake and the amount of energy expended, resulting in an excess of body fat that can affect health. Epidemiological studies have argued that obesity increases the risk of developing asthma, even for low values of excessive weight. Despite knowledge of the association between asthma and obesity, the underlying mechanisms are not yet totally understood. The aim of this study was to evaluate the adipokine levels – resistin, adiponectin and leptin – produced by adipose tissue in overweight and normal weight patients with and without asthma. In this study, 28 overweight asthmatics, 26 overweight non-asthmatics and 26 asthmatics with normal weight were evaluated. Body mass index and abdominal circumference were assessed, skin-prick-tests to allergy performed and serum resistin, adiponectin and leptin measured in all the population. The comparison between the three groups revealed that leptin levels were significantly different, higher in overweight asthmatics. Allergy was associated with higher levels of leptin, but without statistical significance. Adiponectin concentration was very simi-

lar in the three groups, with resistin higher in women with greater abdominal girth. The results indicate a change at the systemic level of adipokines in overweight patients with asthma, particularly in women, with potential impact on respiratory status.

Keywords: Adipokines, adiponectin, asthma, BMI, leptin, obesity, resistin.

RESUMO

Nos últimos anos, tem-se assistido a um aumento da prevalência de asma e obesidade. A asma é uma doença inflamatória crónica das vias aéreas. A obesidade corresponde a um desequilíbrio entre a energia ingerida e a despendida, que resulta em excesso de gordura corporal e em doença. O excesso de peso e a obesidade aumentam o risco de desenvolvimento de asma. Apesar do conhecimento desta associação entre asma e obesidade, os mecanismos subjacentes não estão, ainda, totalmente esclarecidos. O objectivo deste estudo foi avaliar os valores das adipocinas produzidas pelo tecido adiposo – resistina, adiponectina e leptina – em doentes com excesso de peso e com peso normal, com e sem asma. Foram incluídos 28 indivíduos asmáticos com excesso de peso, 26 não asmáticos com excesso de peso e 26 asmáticos com peso normal. Foi calculado o índice de massa corporal, medido o perímetro abdominal, realizados testes cutâneos de alergia por picada e determinada a concentração sérica da resistina, adiponectina e leptina. A comparação dos três grupos revelou que os níveis de leptina são significativamente diferentes, sendo mais elevados nos asmáticos com excesso de peso. A presença de alergia associou-se a valores mais elevados de leptina, mas sem significado estatístico. As concentrações de adiponectina foram mais próximas nos três grupos estudados. A resistina apresentou valores mais elevados nas mulheres com maior diâmetro abdominal. O conjunto de resultados aponta para uma modificação a nível sistémico de adipocinas em asmáticos com excesso de peso, particularmente no sexo feminino, com potencial repercussão na condição respiratória.

Palavras-chave: *Adipocinas, adiponectina, asma, IMC, leptina, obesidade, resistina.*

INTRODUCTION

Asthma affects around 300 million people worldwide and is estimated to cause 250 thousand deaths a year[1]. Asthma is becoming increasingly prevalent[2]. It is a multifactorial disease connected to a raft of environmental and genetic factors[3].

Being overweight or obese is an imbalance between energy intake and the amount of energy expended, in which the intake is greater than the output. This imbalance results in an excess of accumulated body fat and disease, leading to lowered life expectancy and/or more health problems[4] such as type 2 diabetes mellitus, osteoarthritis and chronic cardiovascular and respiratory diseases[5,6]. The factors which determine this imbalance are complex and may be of genetic, metabolic, environmental and behavioural origin.

Obesity and pre-obesity are evaluated by body mass

index (BMI), an index which measures corpulence via dividing weight in kilograms by height in meters squared. The resulting BMI, according to the World Health Organization, divides people into the following categories: underweight (BMI < 18.49 kg/m^2), healthy weight (BMI 18.5–24.99 kg/m^2), overweight (BMI 25–29.99 kg/m^2) and obese (BMI > 30 kg/m^2)[7].

Adipose tissue is an organ which plays a selection of roles, unlike the idea prevalent a decade ago which believed it to be an inert bodily compartment. It performs endocrine tasks by secreting proteins and peptides given the catch-all name of adipokines. In addition it has a vast biological activity ranging from thermal insulation, a physical barrier against trauma, with its main function taken to be storing and releasing energy[4].

There is as yet no consensus as to the possible asthma-obesity association[8]. However, a review of over 40 cross-sectional and case-controlled studies from the 1990s onwards found in almost all an asthma-obesity link[9]. The literature finds that not only the obese but also the overweight have a greater propensity to develop asthma[9]. There are several mechanisms which could contribute to the asthma-obesity association, particularly mechanical factors. Among these are low lung volumes in obese patients which are in turn related to increased bronchial hyperresponiveness and increased elastic load, causing bronchoconstriction, increasing the dyspnoea in these patients. Although mechanical factors play a weighty part in the pathogenesis and clinical presentation of asthma in obese patients[10], other mechanisms are involved in this problem. Among these are systemic inflammation and adipoctye-derived factors such as adipokines, which include resistin, adiponectin and leptin[8]. The action of these proteins and their role in asthma and obesity remain to be elucidated.

Resistin is a protein which contains 108 aminoacids and belongs to the resistin-like molecule family. While resistin's potential function is not completely understood, it has been suggested that it plays a part in the positive regulation of molecular adhesion, with a pro-inflammatory action[8]. This protein is mainly found in macrophages and adipocytes. It has been shown that resistin can induce vascular inflammation via an increased expression of vascular cell adhesion molecule 1 (VCAM-1), inter-cellular adhesion molecule 1 (ICAM-1), and long pentraxin 3 (ptx3) in the vascular endothelium. Exposing macrophages to resistin has been shown to induce expression of tumor necrosing factor-alpha (TNF-α), interleukin (IL)-6 and IL-12, suggesting that resistin is more than a simple marker of inflammation. The relation of concentration of this adipokine in asthma patients is not consensual; some studies show an increased concentration of resistin in these patients' plasma[11] and others indicate a negative relation of the two factors[8].

Adiponectin is a protein involved in glucose and lipid homeostasis and is also an anti-inflammatory adipokine[8] which acts on blood vessel walls and in adipose tissue to inhibit the proliferation of vascular smooth muscle. It also seems to protect the endothelium from the adhesion of macrophages and the damage they cause. At the same time, it seems to increase the oxidation of fatty acids in peripheral tissues, thus preventing ectopic accumulation of fats[12]. Low adiponectin levels have been associated with asthma in population-based studies, but their relevance remains to be elucidated. Obese patients have decreased levels of circulating adiponectin, with the factors contributing to this decrease remaining to be elucidated.

Leptin is a protein which plays a key part in weight regulation, appetite and body energy balance. It also acts on T lymphocytes and their relationship with inflammation in asthma[8], and it has acknowledged pro-inflammatory systemic effects in this pathology. In addition to

adipocyte, leptin is also produced in other human tissues such as the stomach, heart and bronchial epithelium[4]. It also has proliferative and anti-apoptotic effects on the T lymphocytes[13,14]. This protein is increased in obesity. The association between asthma and increased serum leptin remain a subject of controversy, with studies with contradictory results which do not fully explain this association[15].

The aim of this study was to analyse the relationship between asthma and obesity by comparing the levels of three adipokines – resistin, adiponectin and leptin – in overweight and normal weight patients, with and without asthma.

METHODS

This 2010 study was undertaken using an available sample of the population, consisting of 2200 individuals aged 18–74, randomly selected as described in the protocol of the Global Allergy and Asthma European Network (GA^{2}LEN). Potential participants received a questionnaire to fill in and post, with three attempts made to obtain an answer. The questionnaire collected information on age, gender and any asthma symptoms.

A subsample of randomly selected asthma patients underwent skin-prick-tests to allergy using a twelve-allergen battery (birch, grass mix, timothy grass, cat, dog, cockroach, olive tree, artemisia, pellitory, alternaria and house dust mites – *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*). The selection of extracts was made in line with GA^{2}LEN guidelines[16]. Histamine chloride (10 mg/ml) was used as positive control and glycerol saline solution as negative control. The allergen extracts were placed on the inner forearm and the prick performed with Morrow-Brown metal lancets. After twenty minutes, readings were taken of the wheals' mean diameters in millimetres, and tests with wheal diameters over 3mm considered positive.

Following the GA^{2}LEN guidelines, asthmatics were considered those individuals who complained of asthma with characteristic symptoms as detailed in the Global Initiative for Asthma (GINA).

All patients were weighed (clothed but shoeless), their height measured and BMI calculated. Abdominal girth was also measured in the population studied.

This study included both overweight individuals with and without asthma, with BMI over 25 kg/m^2 and normal weight individuals with asthma. All individuals born prior to 1945 were excluded, considered elderly and thus liable to have adipokine abnormalities (unrelated to asthma and overweight).

All participants' informed consent was obtained prior to inclusion in the study, after approval was received from the Ethics Committee of the institution where the study was carried out.

Measuring serum adipokine levels

Measuring serum resistin, adiponectin and leptin was performed using ELISA (Phoenix Pharmaceuticals®, Burlingame, USA) kits. The protocol did not specify the need to dilute the samples, leaving this up to each laboratory. Thus, the resistin was not diluted at all, adiponectin was diluted 1:2 and leptin 1:20. The minimum detection limits of resistin, adiponectin and leptin were 0.016 ng/mL, 0.15 ng/mL and 0.313 ng/mL, in turn.

Statistical analysis

Comparisons were made between groups divided by BMI and asthma diagnoses using the Kruskal-Wallis test as there was abnormal distribution of variables. A $p<0.05$ level of statistical significance was set. We used PASW Statistics 18 and Numbers'09 software.

RESULTS

Three groups were made of the 80 participants selected in the initial sample. These groups followed individuals' BMI and asthma diagnosis:

- Group 1: 28 individuals with asthma and BMI > 25 kg/m^2 (30.4 ± 4.3);
- Group 2: 26 non-asthmatic individuals with BMI > 25 kg/m^2 (28.9 ± 4.2);
- Group 3: 26 individuals with asthma and BMI < 25 kg/m^2 (21.6 ± 1.9).

Table I shows the adipokine levels of Groups 1 and 3.

Resistin, adiponectin and leptin levels in the individuals with positive and negative skin-prick-tests to allergy were 0.71±0.06 *vs.* 0.70±0.06, 23.1±0.5 *vs.* 22.9±1.1 and 72.5±50.3 *vs.* 58±41.4 in turn, with no statistically significant differences found between the groups studied.

In the group with BMI > 25, the differences in the serum levels of the three adipokines in asthmatics (Group 1) and non-asthmatics (Group 2) were not significant (p > 0.05) (Table II).

The serum adipokines in the individuals with BMI > 25 (n=53) were also compared with those with BMI < 25 (n=26). Patients with BMI > 25 had significantly higher levels of serum leptin (Table III).

Resistin, adiponectin and leptin levels in the total population both with and without excess abdominal girth were in turn 0.7±0.05 *vs.* 0.7±0.06 (p not significant), 23±0.95 *vs.* 23.2±0.36 (p not significant),

Table I. Adipokine levels in asthmatics: total and female population

	Total population				Female population		
	Groups	**n**	**Mean value (SD)**	**p**	**n**	**Mean value (SD)**	**p**
Resistin	BMI ≥25 with asthma	23	0,70±0,06	0,456	15	0,73±0,05	0,160
	BMI ≤25 with asthma	21	0,69±0,07		16	0,69±0,06	
	Total	44			31		
Adiponectin	BMI ≥25 with asthma	9	22,9±1	0,587	4	23,2±0,3	0,448
	BMI ≤25 with asthma	6	23,3±0,16		5	23,3±0,17	
	Total	15			9		
Leptin	BMI ≥25 with asthma	28	78,1±44,6	0,006	17	106,7±29,7	<0,001
	BMI ≤25 with asthma	26	39,7±28,3		20	45±29,5	
	Total	54			37		

SD – standard deviation; BMI – body mass index

Table II. Adipokine levels in overweight individuals: asthma and control group

	Groups	n	Mean value (SD)	p
Resistin	BMI ≥25 with asthma	23	0,70±0,06	0,496
	BMI ≥25 no asthma	22	0,70±0,05	
	Total	45		
Adiponectin	BMI ≥25 with asthma	9	23,1±0,4	0,719
	BMI ≥25 no asthma	18	22,9±1	
	Total	27		
Leptin	BMI ≥25 with asthma	28	78,1±44,6	0,943
	BMI ≥25 with asthma	25	78,1±54,6	
	Total	53		

SD – standard deviation; BMI – body mass index

Table III. Adipocin levels in overweight and normal weight individuals: total and female population

	Total population				Female population		
	BMI	n	Mean value (SD)	p	n	Mean value (SD)	p
Resistin	Normal weight	21	0,69±0,07	0,228	17	0,69±0,06	0,074
	Overweight	45	0,71±0,05		26	0,72±0,5	
	Total	66			43		
Adiponectin	Normal weight	6	23,3±0,2	0,338	5	23,3±0,17	0,865
	Overweight	27	23±0,85		11	23,3±0,2	
	Total	33			16		
Leptin	Normal weight	26	39,7±28,3	0,001	21	45,1±28,8	<0,001
	Overweight	53	78,1±49,1		28	110±37,6	
	Total	79			49		

SD – standard deviation; BMI – body mass index

80.6±49.8 *vs.* 51.4±39.6 (p=0.007) and in females with and without excess abdominal girth were in the same order 0.69±0.06 *vs.* 0.73±0.04 (p=0.032), 23.3±0.2 *vs.* 23.3±0.2 (p not significant), 108.1±45.3 *vs.* 62.7±38.2 (p<0.001).

DISCUSSION

Adipokines are protein mediators secreted by adipose tissue. Recently adipokines have also been involved in regulating inflammation and allergic reactions, increasing the risk of asthma, particularly in obese female patients.

In this study we evaluated resistin, adiponectin and leptin, finding higher levels of leptin in overweight asthmatic patients than in those considered normal weight, with this difference more marked in females. In comparing the measurements taken in overweight patients with and without asthma, the differences found were reduced and did not attain statistical significance. Higher leptin levels, however, have been associated with more severe disease, with some authors suggesting the connection between leptin and asthma is not restricted to obesity[17].

Allergy also seems to be related to obesity as it is to asthma. There are positive correlations seen between leptin and IgE levels in patients with allergy, and obesity could be a risk factor for allergy. Allergy is associated with higher levels of this adipokine, but the difference seen did not attain statistical significance, thus seeming to make a modest contribution in these patients, unlike what has been suggested in other studies[18].

The majority of obese individuals had increased leptin, as was seen in other studies, Circulating leptin levels in patients with chronic inflammatory respiratory pathology and with dietetic abnormalities correlates with the levels of leptin in induced sputum[19,20].

High leptin levels could constitute a potential aggravating factor for asthma in overweight patients, particularly in overweight allergic patients.

Resistin is equally an adipokine with a pro-inflammatory role which has been seen to be increased in obesity and several chronic diseases. The group of overweight individuals studied had similar resistin levels to those seen in the normal weight group. The group of females with larger abdominal girth had significantly higher resistin levels.

Adiponectin has anti-inflammatory properties, and is supposedly a positive influence on the regulation of chronic pathologies. Overweight asthmatics have lower levels of this protein, followed by overweight individuals with no pathology and finally by normal weight asthmatics. While this tendency suggests a more limited protective action when the individual is overweight, the similarity of the measurements found in the different groups does not allow for a more detailed discussion of this result.

CONCLUSIONS

The results of this study suggest a modification at the systemic level in the proteins mainly produced by adipocytes in overweight asthmatic individuals. The abnormalities seen were predominantly in leptin and determined by the existence of excess weight and increased abdominal girth in females, with potential impact on the respiratory status.

Our aim in this study was to analyse individuals considered overweight, that is, we used as selection criterion a BMI>25, which probably better corresponds to the Portuguese picture. This fact could have minimised the differ-

ences between groups. This does not mean that the differences seen, particularly in leptin and to a lesser extent in resistin, cannot sustain the persistence of the inflammatory response and consequently of asthma.

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