The influence of apocynin, lipoic acid and probiotics on antioxidant enzyme levels in the pulmonary tissues of obese asthmatic mice

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ABSTRACT

Bronchial asthma and obesity are common health problems. Obesity is already responsible for 300,000 deaths per year.

Aims: The aim of the present study was to assess whether apocynin, alpha lipoic acid and probiotic administration in combination with low-fat diet supplementation influences the levels of antioxidant enzymes in the pulmonary tissues of obese asthmatic mice.

Main methods: The study was performed on male C57/BL6 mice divided into 10 groups: (I) control; (II) asthma; (III) obesity; (IV) asthma + obesity; (V) asthma + obesity + apocynin *p.o.* 15 mg/kg/day for 12 weeks; (VI) asthma + obesity + low-fat diet for 12 weeks; (VII) asthma + obesity + low-fat diet for 12 weeks with apocynin *p.o.* 15 mg/kg/day; (VIII) asthma + obesity + low-fat diet with probiotics for 12 weeks; (IX) asthma + obesity + low-fat diet for 12 weeks with lipoic acid *p.o.* 100 mg/kg/day for 12 weeks; (X) asthma + obesity + standard diet with probiotics for 12 weeks. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) activity were examined. The administration of apocynin alone and apocynin in combination with a low-fat diet resulted in a significant increase in SOD values (respectively $p < 0.001$; $p = 0.010$). Application of probiotics resulted in a decrease in CAT activity ($p = 0.037$) and an increase in GPx activity ($p < 0.001$) compared to obese asthmatic mice. The administration of lipoic acid resulted in an increase in GR activity ($p = 0.024$ *vs.* control).

Key findings: Supplementation containing apocynin, lipoic acid and probiotics has a positive influence on the antioxidant capacity of the pulmonary tissues of obese asthmatic mice.

Significance: These results may contribute to the development of new therapeutic approaches.

1. Introduction

The Global Initiative for Asthma (GINA) [1] reports asthma to be a heterogeneous chronic disease of the respiratory system, affecting 300 million people all over the world [1,2]. Asthma is the most common syndrome in children, and in 2010, 60% of children with asthma reported at least one asthma attack during the previous 12 months. About 250,000 people die prematurely each year from this disease [3–5]. Clinical symptoms *e.g.* breathing problems, shortness of breath or wheezing, occur with particular frequency in the night/morning. Although the condition is known to develop in response to environmental factors such as smoking and air pollution, significant roles can also be played by etiological and genetic factors [6–8].

Many researchers report an increased risk of asthma in obese people, with the term "distinct phenotype of asthma" being used in the literature to describe increased asthma severity and relative corticosteroid resistance. In addition, people suffering from asthma and obesity tend to display significantly higher concentrations of oxidative stress parameters. Of all the organs of the body, the lungs are the most vulnerable to the formation and action of reactive oxygen species (ROS), which requires the presence of oxygen. ROS are produced mainly by the intracellular respiratory and immune processes associated with the inflammatory reaction in response to pathogens [9,10]. NADPH oxidase [11,12], myeloperoxidase (MPO) and xanthine oxidase (XO) play key roles in the endogenous formation of ROS [13–16]. An important role is attributed to NADPH oxidase, which is responsible for the generation of superoxide anions [17] and is thought to function as an antimicrobial agent.

One line of defense against ROS is that of the enzymatic antioxidants, which act by converting radicals to less harmful forms; the most important of these are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [18]. In addition, oxidation can be inhibited by low molecular weight antioxidants known as free radical scavengers; these can be divided into hydrophilic antioxidants, such as ascorbate (vitamin C), glutathione, cysteine, flavonoids and creatinine, and hydrophobic antioxidants, such as vitamin and provitamin D_3, carotenoids, bilirubin, coenzyme Q, tocopherols and cholesterol [19].

The prevalence of obesity increased greatly in recent decades. High-fat diets are known to induce a significant increase in oxidative stress and inflammation in persons with obesity. When obesity persists for a long time, antioxidant sources can be depleted, decreasing the activity of enzymes such as SOD, CAT and GPx. Both radical and non-radical forms of ROS decrease the expression of adiponectin, suggesting that treatment with antioxidants or ROS inhibitors could restore the regulation of adipokines; thus, supplementation with antioxidants would reduce the risk of complications related with obesity and oxidative stress. Obesity causes systemic inflammation due to the chronic release of proinflammatory mediators from adipose tissue. Systemic inflammation may lead to airway inflammation and the development of asthma.

Recent data highlights the involvement of ROS in the pathogenesis of bronchial asthma during obesity. Oxidative damage to cellular components, such as lipids and DNA, results in the occurrence of pathological changes in the respiratory epithelial cells, as well as mucus overproduction or smooth muscle contraction [20–22].

This work focuses on the analysis of antioxidant enzyme activity in the pulmonary tissue of obese asthmatic mice. The results may contribute to the development of new therapeutic approaches.

2. Materials and methods

2.1. Chemicals

Albumin from chicken egg white (lyophilized powder, ≥98%, grade V), acetyl-β methylcholine chloride (≥98% TLC, powder), acetovanillone ≥98%, (±)-α-lipoic acid synthetic, ≥99% (powder) were purchased from Sigma Chemical Co. (St. Louis, MO, USA; ul. Szelągowska 30, 61-626 Poznan, Poland). All other reagents were obtained from Cayman Chemical Company, Ann Arbor, MI. (BIOKOM, ul. Wspolna 3, 05-090 Janki, Poland) and were of analytical grade.

2.2. Animals

Mouse models of chronic asthma were created by ovalbumin (OVA)-challenge (s.c. + aerosol), and mouse obesity models were created by feeding with a high-fat diet for 12 weeks. The study was performed on adult male C57/BL6 mice (n = 7 per group) weighing about 20 g before experiment. The animals were kept in air-conditioned rooms, under standard conditions: 12 h artificial lighting and 12 h the dark at room temperature.

The mice were divided into the following groups: Group 1 (control)-standard-fat diet (for 12 weeks) + injection of saline (days: 0–7–14) 100 μg s.c + exposure to 0.9% of NaCl with a nebulizer; Group 2 - standard diet (for 12 weeks) + injection of ovalbumin (days: 0–7–14) 100 μg s.c + exposure to 1% of OVA with a nebulizer; Group 3 - high-fat diet (for 12 weeks) + injection of saline (days: 0–7–14) 100 μg s.c + exposure to 0.9% of NaCl with a nebulizer; Group 4 - high-fat diet (for 12 weeks) + injection of ovalbumin (days: 0–7–14) 100 μg s.c + exposure to 1% of OVA with a nebulizer; Group 5 - high-fat diet (for 12 weeks) + injection of ovalbumin (days: 0–7–14) 100 μg s.c + exposure to 1% of OVA with a nebulizer + apocynin 15 mg/kg/day for next 12 weeks; Group 6 - high-fat diet (for 12 weeks) + injection of ovalbumin (days: 0-7-14) 100 μg s.c + exposure to 1% of OVA

Table 1
The composition of the used diets.

	Low-fat diet (4% kcal%fat)		High-fat diet (45% kcal%fat)		Standard-fat diet (10% kcal%fat)	
	g	kcal	g	kcal	g	kcal
Protein	19.9	21	25.1	21	20.3	20
Carbohydrates	74.5	75	42.1	34	71.3	70
Lipids	1.9	4	28.3	45	4.6	10

with a nebulizer + low-fat diet for next 12 weeks; Group 7 - high-fat diet (for 12 weeks) + injection of ovalbumin (days: 0–7–14) 100 μg s.c + exposure to 1% of OVA with a nebulizer + low-fat diet + apocynin for next 12 weeks; Group 8 - high-fat diet (for 12 weeks) + injection of ovalbumin (days: 0–7–14) 100 μg s.c + exposure to 1% of OVA with a nebulizer + low-fat diet with probiotics (*L. casei* - 4×10^8 CFU) for next 12 weeks; Group 9 - high-fat diet (for 12 weeks) + injection of ovalbumin (days: 0–7–14) 100 μg s.c + exposure to 1% of OVA with a nebulizer + low-fat diet + lipoic acid (100 mg/kg/day p.o.) for next 12 weeks; Group 10 - high-fat diet (for 12 weeks) + injection of ovalbumin (days: 0–7–14) 100 μg s.c + exposure to 1% of OVA with a nebulizer + standard diet with probiotics (*L. casei* - 4×10^8 CFU) for next 12 weeks (Table 1).

Whole-body plethysmography was performed in all mice (Buxco Small Animal WBP; DSI; Vivari, ul. Pańska 98, 00-837 Warszawa, Poland). At the end of the experimental period, the animals were euthanized. The lungs were then surgically removed, cleaned of extraneous tissue (rinsed with cold isotonic saline), dried by blotting between two pieces of filter paper and weighed on an electronic balance.

The experimental procedures were approved by the Medical University of Lodz Ethics Committee No. 26/ŁB59/2017.

2.3. Determination of SOD activity

The method uses tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine, thus measuring all three SOD types [23]. The SOD Kit (Item No. 706002) comprised Assay Buffer, Sample Buffer, Radical Detector, SOD Standard, Xanthine Oxidase and HPLC-grade water [23].

Briefly, 200 μl of diluted Radical Detector and 10 μl of tissue homogenate were added to each well on the plate; following this, 20 μl of diluted Xanthine Oxidase was added. The 96-well plate was mixed for a few seconds and incubated on a shaker for 30 min at room temperature (Thermo Scientific MaxQ 4000). Absorbance was read at 450 nm with a plate reader (TECAN Sunrise with software Magellan Standard).

2.4. Determination of CAT activity

The method is based on the reaction of an enzyme with methanol in the presence of H_2O_2. The produced formaldehyde forms a bicyclic heterocycle with aldehydes and can be measured colorimetrically with 4-amino-3-hydrazino-5mercapto-1,2,4-triazole as chromogen [24]. The CAT Kit (Item No. 707002) consisted of the following: Catalase Assay Buffer, Catalase Sample Buffer, Catalase Formaldehyde Standard (4.25 M formaldehyde), Catalase – Control, Catalase Potassium Hydroxide, Catalase Hydrogen Peroxide, Catalase Purpald, Catalase Potassium Periodate, methanol and HPLC-grade [24].

Each well on the plate was filled with diluted Assay Buffer (100 μl), methanol (30 μl) and tissue homogenate (20 μl). Next, 20 μl of hydrogen peroxide was added to all used wells and the plate was incubated on a shaker for 20 min at room temperature (Thermo Scientific MaxQ 4000). Following this, 30 μl of potassium hydroxide was added. The 96-well plate was mixed and incubated on a shaker for 10 min at room temperature with 30 μl of Catalase Purpald. The solution was finally
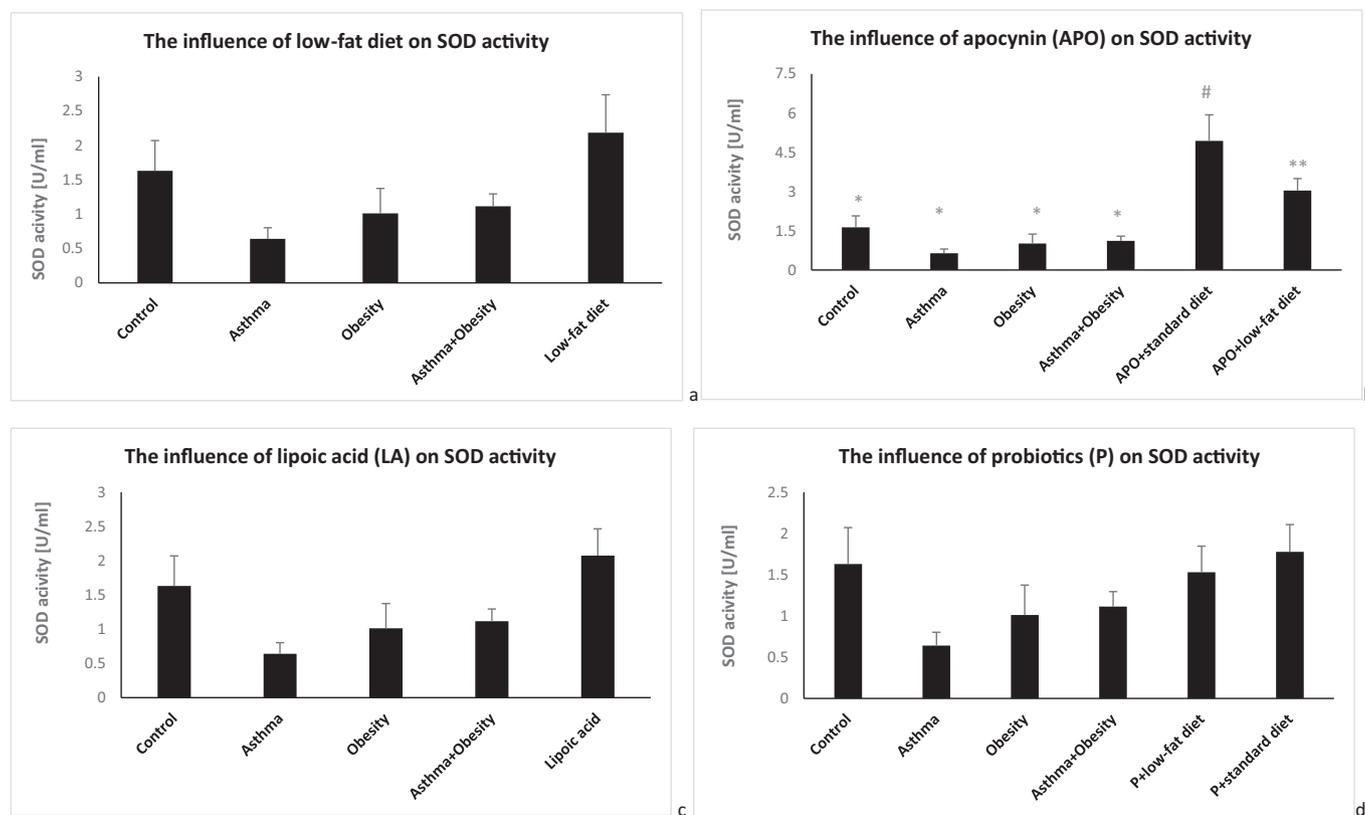
Fig. 1. The influence of low-fat diet (a), apocynin (b), lipoic acid (c) and probiotics (d) on the SOD activity in all groups of animals. Mice treated with apocynin (APO) alone displayed higher SOD activity than the obese, asthmatic and obese + asthmatic mice. Those receiving APO + low-fat diet demonstrated greater SOD activity than asthmatic, obese and obese + asthmatic mice. Data is shown as mean ± S.E.M.*p < 0.001 vs. APO group; #p = 0.006 vs. APO + low-fat diet group; **p = 0.01 vs. asthma + obesity group.

incubated with 10 μl of potassium periodate for 5 min. The absorbance was read at 540 nm.

2.5. Determination of GPx activity

The assay works by converting glutathione disulfide to its reduced state with GR and NADPH: oxidation of this enzyme results in a decrease in absorbance [25]. GPx Kit (Item No. 703102) consisted of the following: GPx Assay Buffer, GPx Sample Buffer, Glutathione Peroxidase - Control, GPx Co-Substarate Mixture and GPx Cumene Hydroperoxide [25].

Briefly, Assay Buffer (100 μl), GPx Co-Substrate Mixture (50 μl) and sample (20 μl) were added to the sample wells. Next, GPx Cumene Hydroperoxide (20 μl) was added and the plate was carefully mixed for a few seconds. Absorbance was read every minute at 340 nm to obtain readings at five time points.

2.6. Determination of GR activity

The method determines GR activity by measuring the rate of oxidation of NADPH to NADP+, which is related to absorbance at 340 nm [26]. The GR Kit (Item 703202) consisted of GR Assay Buffer, GR Sample Buffer, GR GSSG, GR glutathione reductase-Control and GR NADPH. Briefly, Assay Buffer (100 μl), GSSG (20 μl) and sample (20 μl) were added. Next, 50 μl of NADPH was added to all used wells. The 96-well plate was carefully mixed for a few seconds. Absorbance was read every minute at 340 nm at five time points [26].

2.7. Statistical analysis

The results are presented as mean ± the standard error of the mean

(SEM). The statistical significance was evaluated by ANOVA followed by Duncan's multiple range test as a post hoc test or Dunn's test.

3. Results

3.1. Evaluation of SOD activity

Fig. 1(a–d) shows the changes of SOD activity in lung homogenates. Mice treated with apocynin alone displayed higher SOD activity than the asthmatic, obese and obese + asthmatic animals (p < 0.001). In addition, those receiving apocynin in combination with a low-fat diet demonstrated greater SOD activity than asthmatic, obese and obese + asthmatic mice (p = 0.006 vs. APO + standard-fat diet group).

SOD activity was also insignificantly higher in the low-fat diet group, lipoic acid + low-fat diet group, probiotics + standard diet group and probiotics + low-fat diet group.

3.2. Evaluation of CAT activity

Mice administered α-lipoic acid displayed an insignificant increase in CAT activity in lung tissue compared to controls. Those receiving probiotics displayed lower CAT activity than the obese asthmatic animals (p = 0.037). In the probiotics + low-fat diet group, CAT activity also fell insignificantly. The groups treated with apocynin or apocynin + low-fat diet or lipoic acid exhibited insignificantly lower CAT activity than the asthma + obesity group. The changes of CAT activity in the pulmonary tissue of obese asthmatic mice are given in Fig. 2(a–d).

3.3. Evaluation of GR activity

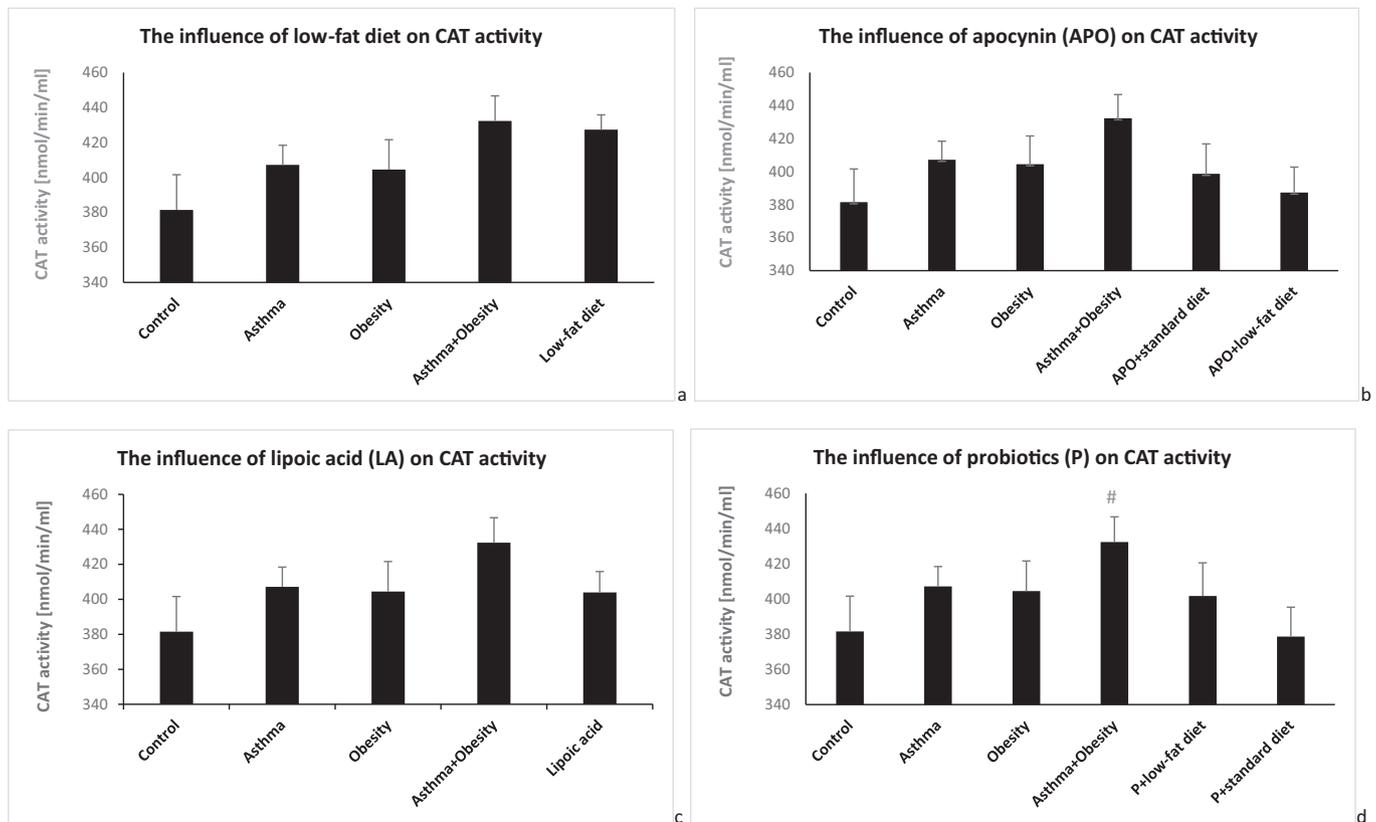Treatment with lipoic acid (plus low-fat diet) significantly increased

Fig. 2. The influence of low-fat diet (a), apocynin (b), lipoic acid (c) and probiotics (d) on the CAT activity in all groups of animals. Mice administered α-lipoic acid (LA) displayed an insignificant increase in CAT activity in lung tissue *vs.* control. Those receiving probiotics displayed lower CAT activity than the obese asthmatic animals. Data is shown as mean ± S.E.M. #p = *0.037 vs. P + standard diet group.*

GR activity compared with the asthma group (p = 0.019), obesity group (p = 0.023) and control group (p = 0.024). The level of GR activity increased in the low-fat diet and probiotics + standard diet groups. However, no significant changes were observed. An insignificant decrease in GR activity was observed in the apocynin and apocynin + low-fat diet groups *vs.* asthma + obesity (Fig. 3a–d).

3.4. Evaluation of GPx activity

The administration of probiotics resulted in increase in GPx activity (p < 0.001). A non-significant increase in GPx activity was observed in the apocynin and the apocynin + low-fat diet group. The level of GPx activity increased also in the lipoic acid group and the low-fat diet group. However, no significant changes were observed (Fig. 4a–d).

3.5. Evaluation of mouse body weight and respiratory parameters

Body weight increased in all groups during the experiment (Fig. 5a–b). The mice fed a high-fat diet (45%kcal%fat) gained weight more rapidly than those fed a standard diet (10%kcal%fat). The final body weight of high-fat diet groups: obesity and asthma + obesity was significantly higher than mice of standard-diet groups: control and asthma (p < 0.001). Mice fed a diet consisting of 45% fat weighed nearly 40% more than mice fed a 10% fat diet for the same time period.

The mice fed a low-fat diet (4%kcal%fat) and those fed a standard diet with apocynin and probiotics quickly lost body weight (p < 0.001). Mice fed a diet consisting of 4% fat weighed nearly 25% less than when they received a high-fat diet.

Whole-body plethysmography (WBP) was performed in all mice. This technique avoids the use of anesthesia and restraints. The degree of bronchoconstriction was expressed as enhanced pause (PenH). The

PenH value was significantly higher in the asthma group and asthma + obesity group than the control group (respectively p = 0.024, p = 0.006). The PenH value in APO and LA groups was lower than the asthma + obesity group (Fig. 6).

4. Discussion

The prevalence of asthma is steadily increasing, and by 2025, the number of cases is expected to have grown by another 100 million (WHO). Recently, "a distinct phenotype of asthma" has been described, which is directly related to the co-occurrence of obesity, and is characterized by increased asthma severity and relative corticosteroid resistance.

Increased body fat content is known to be associated with excessive contents of oxidative stress markers (OS) in the blood. However, the mechanism behind the effect of obesity-related OS on respiratory diseases is not fully understood. It is known that the overproduction of ROS increases the secretion of proinflammatory cytokines such as IL-6 in endothelial cells, and activates the production of TNF-α, IL-6, IL-8 in macrophages and monocytes. ROS are generated in the lungs by NADPH oxidase, xanthin oxidase and nitric oxide synthase pathways. Protein kinase C (PKC) regulates the activity of NADPH oxidase. Oxidative stress leads to excessive production of ROS, which may participate in the pathogenesis of asthma in obese patients. It was found that the adipose tissue of obese mice displayed elevated ROS production accompanied by increased expression of NADPH oxidase and decreased expression of antioxidative enzymes.

Obesity induces systemic inflammation through the chronic release of proinflammatory mediators from adipose tissue. Systemic inflammation may lead to airway inflammation and the development of asthma. Low-fat diet supplementation with antioxidants would reduce
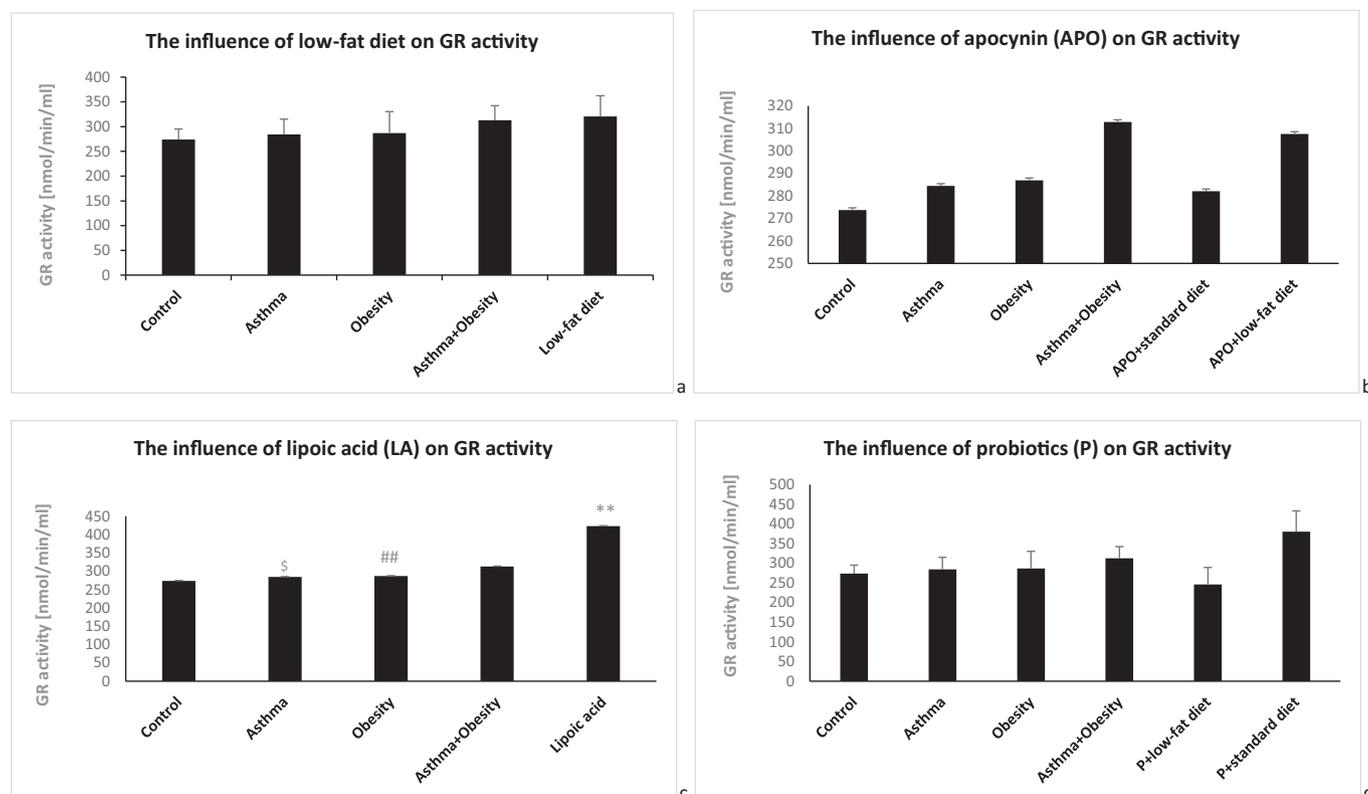
Fig. 3. The influence of low-fat diet (a), apocynin (b), lipoic acid (c) and probiotics (d) on the GR activity in all groups of animals. Treatment with LA + low-fat diet significantly increased GR activity *vs.* asthma group, obesity group and control group. The level of GR activity increased in the low-fat diet and probiotics + standard diet groups. An insignificant decrease in GR activity was observed in the APO + standard diet and APO + low-fat diet groups *vs.* asthma + obesity group. Data is shown as mean ± S.E.M. **p = *0.024 vs. control*; $^{\$}$p = *0.019*; $^{\#\#}$p = *0.023 vs. LA group*.

the risk of complications related with obesity and oxidative stress.

Our findings indicate that the administration of apocynin, lipoic acid and probiotics caused a significant increase in selected antioxidant activity of pulmonary tissues in obese asthmatic mice. The enzymatic defense system converts radicals to less harmful forms [19]. Within this system, the first line of defense is SOD, which acts by breaking down the superoxide anion into hydrogen peroxide and oxygen. SOD can be associated with the cell membrane or can exist in a free form in the plasma. It is known to exist in three forms in the body: CuZnSOD, present in the cytoplasm, MnSOD, present in the mitochondrial matrix and EC-SOD, present in extracellular fluids. All three types of SOD show high expression in lung tissues [27,28].

CAT is found in the cytoplasm, where it breaks down harmful H_2O_2 into oxygen and water. The enzyme is expressed by type II follicular cells and macrophages [29,30]. GPx decomposes H_2O_2 to water, and reduced glutathione (GSH) to glutathione disulfide (GSSG). Glutathione reductase (GR) is responsible for maintaining an adequate level of reduced glutathione and acts by reducing GSSG to GSH [31]. Glutathione is one of the most common thiol compounds in nature. It is synthesized in the cytoplasm from cysteine, glycine and glutamic acid. It exists in several redox forms, the most important of these being reduced glutathione and oxidized glutathione, with the ratio of reduced to oxidized glutathione being an indicator of oxidative stress.

GSH plays a role in electrophile and oxidant detoxification through enzymatic reactions involving glutathione peroxidase and glutathione-S transferase (GST). Moreover, GSH plays a key role in the regulation of subcellular and cellular redox state or vitamin C or E regeneration. However, its main function is maintaining protein thiol groups in a reduced state to ensure their activity. Glutathione also enables the excretion of xenobiotics (GSX) by binding to them and increasing their hydrophilic properties [32,33].

Overproduction of ROS has been found to reduce the antioxidant activity of SOD, CAT, GR and GPx in asthmatic patients [34]. Two recent works [35,36] describe the rapid loss of activity of SOD during antigen-induced asthmatic response. Enzyme activity was lower in the serum of asthmatic subjects *vs.* control, and activity loss was related to airflow limitation. In addition, CAT and SOD activity have been reported to be significantly lower in the blood of children suffering from asthma, even during resting conditions [37]. Several studies [38] confirm that SOD activity is lower among asthmatic patients compared to controls. Tekin et al. report no significant difference in GPx and CAT activities between controls and asthmatic patients [38]. Increased SOD activity and decreased GPx activity has been reported in the RBCs and leukocytes of patients; however, the CAT activity in RBCs was not altered [39]. Other researchers [40] report no differences between any of the study groups with regard to SOD and GPx activity.

Many recent studies have examined the effects of oxidative stress (OS) on the development of bronchial asthma [41–45]. It has been found that overproduction of ROS results in increased total oxidant status as well as reduced SOD, CAT, GPx activity and total antioxidant status in asthmatic patients [34]. Other OS parameters identified in the course of asthma in recent years were malondialdehyde (MDA) and isoprostane (IsoP) concentrations, total oxidant concentration and myeloperoxidase level [46]. The concentrations of H_2O_2 and NO in exhaled breath condensate of asthmatic patients have also been determined [47–49].

Oxidative stress can also be reduced by exogenous antioxidants, such as vitamins C and E, or phenolic substances such as tocopherols and flavonoids. Administration of α-tocopherol at a dose of 0.1 mg/dl has been found to result in a 5% decrease in the risk of asthma [42]. SOD activity has also been found to protect against asthma development. It has also been found that asthma patients demonstrated lower
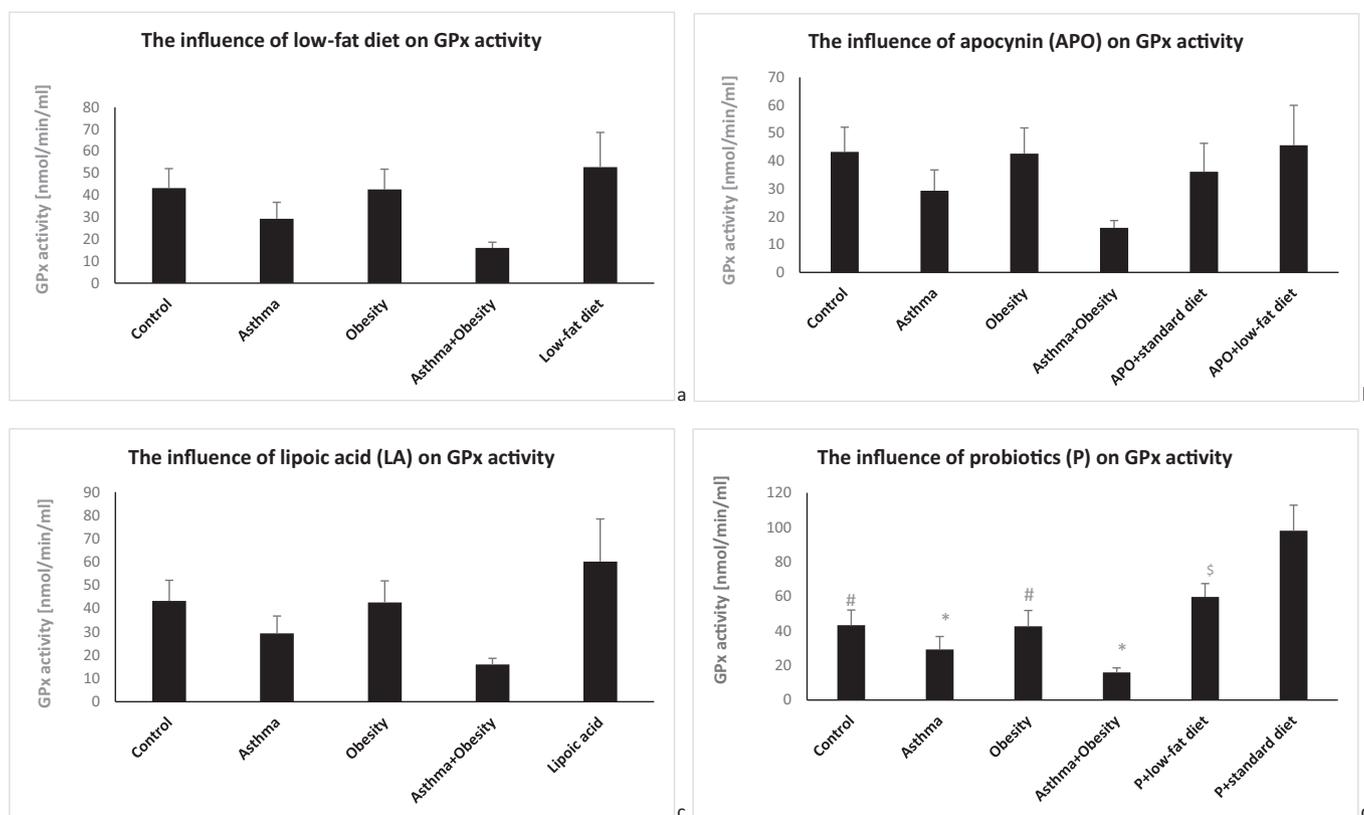
Fig. 4. The influence of low-fat diet (a), apocynin (b), lipoic acid (c) and probiotics (d) on the GPx activity in all groups of animals. The administration of probiotics (P) resulted in increased GPx activity. A non-significant increase in GPx activity was observed in the APO + standard diet, APO + low-fat diet, LA and low-fat diet groups. Data is shown as mean ± S.E.M. *p < 0.001; #p = 0.006; $p = 0.032 vs. P + standard diet group.
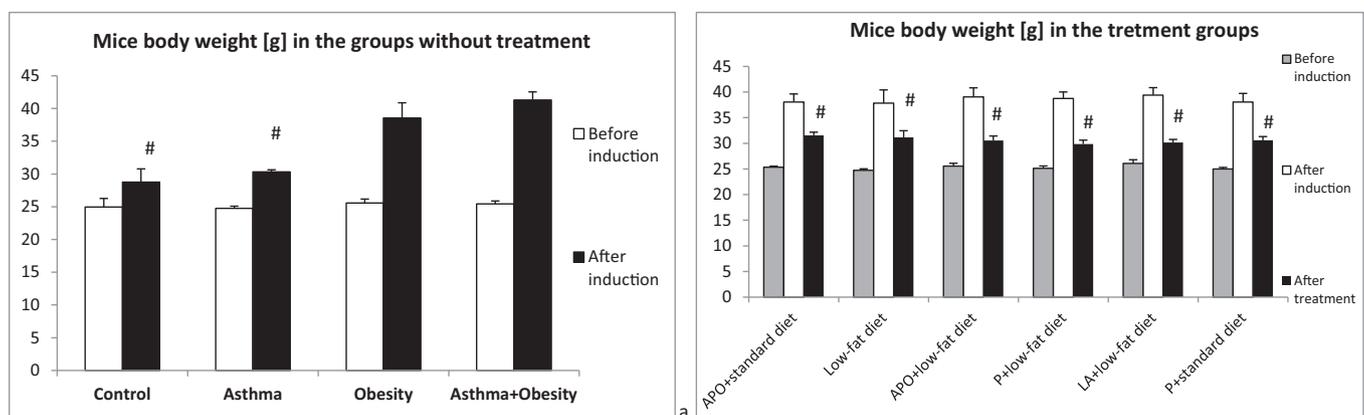

Fig. 5. Body weight increased in all animals of all groups over the course of the study (a - without treatment; b- with the intervention). The mice fed a high-fat diet gained weight more rapidly than mice fed a standard diet. The final body weight of high-fat diet groups (obesity and asthma + obesity groups) was significantly higher than mice of standard-diet groups (control and asthma) (#p < 0.001; Duncan's Method). The mice fed a low-fat diet and standard diet with apocynin, LA and probiotics lost body weight over the next twelve weeks (p < 0.001; Duncan's Method). Data is shown as mean ± S.E.M.

plasma α-tocopherol levels than controls [50].

Many researchers have examined the concentrations of antioxidant vitamins in asthmatic patients. Among children, vitamin A deficiency may have etiological implications in asthma [51], as well as low vitamin C level [52]. A meta-analysis by Allen et al. [53] confirmed that low dietary intakes of vitamins C and A are associated with a significantly higher chance of asthma and wheezing.

The aim of the present study was to determine the influence of apocynin treatment, with or without a low-fat diet, on the levels of antioxidant enzymes in the pulmonary tissues of obese asthmatic mice. NADPH oxidase is the major enzyme responsible for ROS generation in

the lungs. This enzyme generates superoxide anions by transferring electrons from NADPH inside the cell across the membrane and coupling these to molecular oxygen to produce superoxide anion. Apocynin possesses powerful antioxidant and anti-inflammatory properties, associated with its ability to block the activity of NADPH oxidase; this is achieved by interfering with the binding of cytosolic NADPH oxidase components to its membrane components. Apocynin is known to be one of the most important scavengers of H_2O_2, and recent studies suggest it may also have neuroprotective properties [54].

In our study, the administration of apocynin alone and apocynin in combination with a low-fat diet resulted in a significant increase in SOD
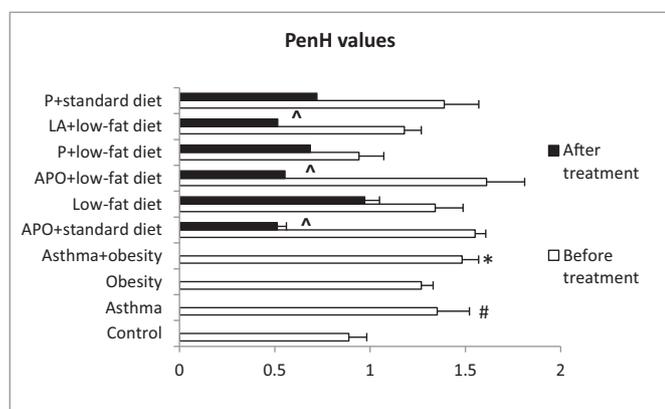
Fig. 6. Whole-body plethysmography was performed in all mice. The PenH value was significantly increased in the asthma group and the asthma + obesity group compared with controls. The PenH value in APO and LA groups was significantly lower than the asthma + obesity group. Data is shown as mean ± S.E.M. $^{\#}p = 0.024$; $^{*}p = 0.006$; $^{\hat{}}p < 0.05$.

values. Nesi et al. [55] noted that apocynin ameliorated airway hyperresponsiveness; it also reduced IL-4 and IL-13 concentrations, as well as eosinophil peroxidase activity. It has also been demonstrated that apocynin significantly attenuates ovalbumin (OVA)-induced AHR and inflammation. It is known to act as a NADPH inhibitor, and may attenuate the influx into bronchoalveolar lavage fluid of inflammatory cells and soluble products, such as IL-4, IL-5, IL-12, IL-13, macrophages, eosinophils and TNF-α. Apocynin may be useful in the treatment of inflammatory diseases induced by OS through NOX activity; it has been found to increase IL-1β secretion and abrogate leukocyte peribronchiolar infiltrate [54]. Administration of apocynin, during intermittent hypoxia (IH) exposure has also been found to attenuate IH-induced increases in hepatic MDA [56]. Peters et al. [57] report that apocynin reduced ozone-induced AHR in mild asthmatics. Further studies on the effect of apocynin on asthma exacerbations in regions with high levels of ambient summer air pollution are needed.

Our results show that administration of α-lipoic acid (LA) resulted in an insignificant increase in the CAT activity of mouse lung tissue *vs.* control. Treatment with lipoic acid (together with a low-fat diet) significantly increased GR activity compared with the control, asthma and obesity groups. LA has strong antioxidant properties and is known to possess therapeutic potential; in fact, it is also known as "an antioxidant of antioxidants". Its reduced form, dihydrolipoic acid (DHLA), can easily quench radicals and chelate metals; in addition, it also has an amphiphilic character and does not exhibit any serious side effects. It has been applied in the treatment of many diseases [58,59]. The present study aims to assess the effectiveness of LA in the treatment of asthma.

Several studies confirm that LA can play a role in asthma pretreatment. Park et al. note that α-lipoic acid (LA) inhibits the activation of Nrf2 and NF-κB, resulting in the attenuation of allergen-induced airway remodeling [60]. Another study [61] examining the effect of choline and alpha-lipoic acid on oxidative stress in a mouse model of airway disease found that LA treatment suppressed TNF-alpha level significantly *vs.* ovalbumin-challenged mice.

Increased ROS generation by bronchoalveolar lavage (BAL) fluid cells has been observed in an ovalbumin-induced murine model of asthma [62]. Increased expression of IL-18, both protein and mRNA, bronchial hyperresponsiveness, airway inflammation and ROS levels were reduced by administration of alpha-lipoic acid: IL-18 expression may also be regulated by ROS, while it may be reduced by antioxidants in asthma through the inhibition of NF-kB [62]. Cho et al. [63] confirm that OS plays an important role in asthmatic airway inflammation and that LA may be useful as adjuvant therapy for bronchial asthma. Animals treated with LA had significantly reduced AHR and smaller

proportions of eosinophils (BAL cells). LA treatment also reduced IL-4 and IL-5 concentrations in BAL fluid, lowered serum OVA-specific IgE concentrations and decreased the DNA-binding activity of NF-kB.

The potential effects of probiotics on the lung microbiome are unknown. In this work, 12-week supplementation of probiotics with or without a low-fat diet might was found to potentially have a positive influence on the antioxidant capacity of pulmonary tissues of obese asthmatic mice. Several studies discuss the application of probiotics in asthma pretreatment, with OS protection being another potential beneficial effect of probiotic supplementation. The most widely studied strain is *Lactobacillus*. Probiotics may act directly on the neutralization of oxidants by the expression of antioxidant enzymes.

Probiotic administration increased the activity of SOD and GPx in obese asthmatic mice. These findings are consistent with recent studies. Nunes et al. confirm that probiotic pretreatment during neonatal age provides protection against asthma through the modulation of microbiota and T cells: the authors describe correlations between qualitative features inherent to neonatal cells, healthy microbiota and T cells [64]. One-year long-term perinatal probiotics administration demonstrated a beneficial effect on the development of eczema up to the age of two years [65]. Other authors [66,67] note that *B. lactis*, *B. bifidum* and *L. lactis* have good IL-10-inducing capacity and may significantly inhibit the activity of the Th2-related cytokines IL-5 and IL-13. Selected probiotics reduced the development of eczema up to the age of two years; however, these beneficial effects extend to preventing the development of primary asthma. Moreover, *Lactobacillus* GG was found to reduce the concentration of exhaled NO among 4- to 7-year-olds [68].

In contrast to these findings, Krzych-Fałta et al. [69] suggest that probiotics have no protective effect on allergy development; however, supplements such as kefir and yogurts administered several times a week in late adolescence showed health-promoting effects in allergic diseases. Another study reports that no tested probiotic products (*L. rhamnosus*, *L. helveticus*) demonstrated any preventive effects against any allergic disorders in early childhood [64]. Other studies [65,70,71] found that early administration of probiotics (*L. reuteri*, *L. rhamnosus* HN019 or *L. paracasei* spp. F19) to infants did not result in a lower prevalence of allergic disease; however, the probiotic L. *rhamnosus* HN001 may have potential value in preventing the development of eczema [72], suggesting that benefits may be species specific. Further studies are needed to assess the effectiveness of potential probiotics with regard to their potential to prevent allergic diseases.

ROS induce a variety of pathologic changes in the airways, one of which is increased reactivity. Increased oxidative stress, either systemic or restricted to the airway, may be a potential mechanism by which obesity can exacerbate asthma. However, more research is needed to confirm this thesis.

5. Conclusion

OS develops when there is an imbalance between production and the elimination of ROS. Our present findings indicate a significant decrease in the activity of antioxidant enzymes (SOD, GPx) among asthmatic mice, suggesting that the presence of asthma is associated with excessive levels of oxidative stress markers in the blood. The administration of apocynin, either alone, in combination with a low-fat diet, with probiotic supplementation, or with both probiotics and a low-fat diet, resulted in a significant increase in SOD, GPx and GR values compared to controls. Supplementation containing apocynin, lipoic acid and probiotics might have a positive influence on antioxidant capacity of pulmonary tissues in obese asthmatic mice. It is likely that ROS are mainly generated in the lungs though the NADPH oxidase pathway.

Declaration of competing interest

There is no conflict of interest.

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