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Dietary supplementation with soybean lecithin increases pulmonary PAF bioactivity in asthmatic rats☆

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Abstract

The prevalence of asthma has risen over the last few decades, and some studies correlate this with the greater consumption of polyunsaturated fatty acids (PUFAs). Dietary PUFAs are known to increase the susceptibility of biological structures to lipid peroxidation, a process by which platelet-activating factor (PAF)-like lipids can be generated. These lipids functionally mimic the bioactivity of PAF, a potent proinflammatory mediator that exerts several deleterious effects on asthma. Thus, this work aimed to investigate if dietary supplementation with soybean lecithin (SL), a source of PUFAs, increases lipid peroxidation and PAF bioactivity in lungs of asthmatic Wistar rats. Animals were separated into groups: control, supplemented, asthmatic, asthmatic supplemented with SL (2 g/kg body weight), asthmatic supplemented with SL (2 g/kg body weight) and DL-α-tocopheryl acetate (100 mg/kg body weight). Asthmatic inflammation increased pulmonary lipid peroxidation, PAF bioactivity, alveolar–capillary barrier permeability and production of nitric oxide. In asthmatics, dietary supplementation with SL promoted an increase in pulmonary lipid peroxidation and PAF bioactivity, and an increase in the permeability of the alveolar–capillary barrier. Moreover, the treatment of asthmatic rats with DL-α-tocopheryl acetate inhibited the lipid peroxidation and decreased the PAF bioactivity. Therefore, the increase in pulmonary PAF bioactivity in asthmatic individuals elicited by the dietary supplementation with SL probably involves the generation of PAF-like lipids. This finding suggests that PAF-like lipids may account for the deleterious effects of dietary PUFAs on asthma.

Keywords: Asthma; Peroxidation; PUFA; Lecithin; PAF-like

1. Introduction

The prevalence of asthma has markedly increased in developed countries over the last few decades [1], in contrast to a relatively stable prevalence of this disease in developing countries over the same period [2]. Indeed, the westernized lifestyle, a characteristic of developed countries, plays a role in the pathophysiology of asthma [3,4] and, among factors associated with this lifestyle, the recent dietary change is a potential contributor to the increase in the prevalence of asthma [1].

An increase in the ratio of dietary polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFA), named P/S ratio, has been observed in westernized diet over the last few decades [1,5,6]. This event is associated with asthma susceptibility, as demonstrated by some studies that related consumption of PUFA with higher, and consumption of SFA with lower susceptibility to asthma [7-10]. It was hypothesized that an increase in n-6 PUFA/n-3 PUFA ratio, instead of the increase in P/S ratio, could raise the susceptibility to the development of asthma [5], but this hypothesis is not supported by epidemiological studies [1]. On the other hand, the increase in P/S ratio is related with higher susceptibility of the biological structures to lipid peroxidation [11,12]. Due to the fact that asthmatic inflammation leads to the production of oxygen and nitrogen reactive species that overcome the host antioxidant defenses [13-15] and thus causes oxidative stress, lipid peroxidation occurs and exerts deleterious effects on asthma [16].

The peroxidation generally occurs in PUFAs esterified on phospholipids [17] and can be followed by reactions that nonenzymatically produce proinflammatory mediators [16]. One of these reactions is the cleavage of a carbon–carbon bond of PUFA sterified at the *sn*-2 position of the phosphatidylcholine glycerol backbone, generating lipids with platelet-activating factor (PAF) activity named PAF-like lipids [18]. The PAF-like lipids mimic the biological activity of PAF [18], one of the most potent and versatile proinflammatory mediators [19], generated enzymatically and structurally identified as 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine [20].

PAF exerts various proinflammatory effects that intensify the asthmatic inflammation [21-23] even in sub-nanomolar concentrations

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[19]. Thus, in asthmatic individuals, dietary supplementation with PUFAs could increase both the lipid peroxidation and the pulmonary PAF bioactivity — possibly by increasing the generation of PAF-like lipids — influencing the asthmatic inflammation. Therefore, this study investigated if dietary supplementation with soybean lecithin, rich in PUFA-containing phosphatidylcholine, increases the pulmonary PAF bioactivity in asthmatic rats.

2. Materials and methods

2.1. Supplements and reagents

DL-α-tocopheryl acetate (Microvit E promix 50) was courtesy from Adisseo (Adisseo Brasil SA, France). Soybean lecithin was donated by Herbarium (Colombo, Brazil). BN52021 (Gingkolide B) was obtained from Biomol (Plymouth Meeting, PA). Fura-2 AM (Cat. No. F-0888), PAF (β-acetyl-γ-*O*-hexadecyl-L-α-phosphatidylcholine, P-4904) and ovalbumin (OVA; Grade II, A-5253) were obtained from Sigma (St. Louis, USA). All others chemicals were purchased from Sigma.

2.2. Animals and supplementation

Adult male Wistar rats (*Rattus norvegicus albinus*), weighing 220±30 g, were obtained from the Biological Sciences Sector of the Federal University of Paraná (Curitiba, Brazil). Rats were allowed free access to water and food (52% of carbohydrates, 21% of proteins and 4% of total lipids; Nuvilab CR1, Nuvital Nutrientes Ltda, Curitiba, Paraná, Brazil), and were maintained at 12 h inverted light/dark cycle. After 10 days of acclimatization under these conditions, the rats were randomly divided into five groups: control (C), supplemented with soybean lecithin (S), asthmatic (A), asthmatic supplemented with soybean lecithin (AS), asthmatic supplemented with soybean lecithin and DL-α-tocopheryl acetate (ASE). The protocol of dietary supplementation consisted of daily oral administration, for 21 consecutive days, of tap water (2 ml/kg body weight) to Groups C and A, soybean lecithin (2 g/kg body weight) to Groups S and AS, soybean lecithin (2 g/kg body weight) and DL-α-tocopheryl acetate (100 mg/kg body weight) to Group ASE. Protocols were approved by the Animal Experimentation Ethics Committee of Federal University of Paraná, in accordance with Brazilian College on Animal Experimentation (COBEA) guidelines.

2.3. Lipid composition of soybean lecithin

Soybean lecithin consists of phosphatidylcholine 31.7%, phosphatidylethanolamine 20.8%, phosphatidylinositol 17.5%, phosphatidylserine 3.0%, phosphatidic acid 2.0% and other phospholipids 10.2%. The fatty acid composition is linoleic acid 64.0%, palmitic acid 14.0%, oleic acid 10.0%, α-linolenic acid 7.0% and stearic acid 4.0%.

2.4. Asthma induction

This protocol was based on the work of Schuster et al. [24], with some modifications. At the 4th day of the dietary supplementation protocol, individuals from Groups A, AS and ASE were sensitized with 1 ml of phosphate-buffered saline solution containing 1 mg of OVA and 200 mg of $Al(OH)_3$, injected subcutaneously. This procedure was repeated 7 days after the first sensitization. After another 7 days, the pulmonary allergen challenge was performed. For that, the animals were placed during 30 min in a 3 dm^3 chamber connected to a nebulizer which delivered an aerosol of 5% OVA in PBS, at 20 ml of solution per hour. This allergen challenge was repeated for three consecutive days. Groups C and S received subcutaneous application of 1 ml PBS containing 200 mg $Al(OH)_3$ during the two steps of sensitization and were exposed to an aerosol of PBS, at the same conditions described for Groups A, AS and ASE. Twenty-four hours after the last allergen challenge, coinciding with the end of the dietary supplementation protocol, the bronchoalveolar lavage fluid (BALF) and the lungs of the animals were collected as described below.

2.5. BALF collection and processing

Rats were given a lethal dose of urethan (3 mg/g) intraperitoneally. The BALF was then collected as described by Schuster et al. [24]. Trachea was cannulated and, using a syringe, the lungs were gently lavaged with portions of 5 ml PBS (4°C). The fluid was retrieved by gentle aspiration and maintained in a centrifuge tube placed in ice bath. This procedure was repeated 10 times for each animal, and a 300-μl sample of the first retrieved fluid was centrifuged to obtain the cell-free BALF, which was then stored at −20°C for further total proteins and nitrite dosages. The remaining BALF was pooled, centrifuged (290×*g*, 8 min, 4°C) and the cell pellet was resuspended in 0.5 ml PBS for differential cell count. As determined by light microscopy analysis (at ×1000 magnification) after May-Grünwald/Giemsa staining, the percentage of eosinophils in BALF was significantly higher in asthmatics when compared with non-asthmatics, a characteristic of the asthmatic inflammation expected for this model.

2.6. Lung collection

After BALF collection, rats were thoracotomized and PBS was injected into the heart right ventricle to remove the blood from pulmonary vessels. Then, the lungs were collected, washed in PBS and placed in ice. A portion of the lower pulmonary right lobe was collected for immediate measurement of the lipid hydroperoxide concentration and the catalase activity. The remaining tissue was stored at −80°C for further measurement of PAF activity.

2.7. Total lipid extraction

The method described by Folch et al. [25] was performed for total lipid extraction. A 70-mg portion of the lower pulmonary right lobe was homogenized in 1.33 ml of methanol/chloroform (1:2, v/v) using an electric homogenizer (GGS 27, Bosch). The homogenate was added with 480 μl methanol and the mixture was then centrifuged (5000×*g*, 10 min, 4°C). The supernatant was transferred to a centrifuge vial (1.5 ml) and added with 960 μl of chloroform. The extract was then washed once with ultrapure water and three times with washing solution (1.8 mM $CaCl_2$, 1.8 mM $MgCl_2$, 49.7 mM NaCl, 3.0% chloroform, 49.0% methanol, 48.0% ultrapure water). After that, the extract was dried with a nitrogen stream at 37°C and stored at −80°C under nitrogen atmosphere.

2.8. BALF total proteins

The method described by Bradford [26] was carried out for this measurement. Briefly, 250 μl Bradford reagent was added to 10 μl of cell-free BALF in a microplate. After 5 min at room temperature, absorbance at 595 nm was measured using a microplate spectrophotometer (Benchmark, Bio-Rad). Protein concentration was determined by interpolating absorbance values in a standard curve generated by known concentrations of bovine serum albumin. Results were expressed as micrograms of total proteins per milliliter of BALF.

2.9. BALF nitrite

This assay was carried out according to Stuehr and Marletta [27]. In a 96-well microplate, 100 μl of Griess reagent (1:1 mixture of 1% sulfanilamide in 5% orthophosphoric acid and 0.1% *N*-[1-naphthyl]ethylenediamine) was added to 100 μl cell-free BALF. Five minutes after addition of Griess reagent, absorbance was measured at 550 nm using a microplate spectrophotometer (Benchmark, Bio-Rad). Results were expressed as percentage of BALF nitrite relative to group control.

2.10. Pulmonary catalase activity

Catalase activity was measured in extract of the pulmonary tissue using the method described by Aebi [28]. A 50-mg portion of the lower pulmonary right lobe was homogenized in 2 ml PBS using an electric homogenizer (GGS 27, Bosch). After centrifugation (10,600×*g*, 20 min, 4°C) to remove the particulated matter, the extract was placed in ice bath. A 10-μl aliquot of this pulmonary tissue extract was added to 990 μl of reactive medium (20 mM hydrogen peroxide, 0.05 M tris[hydroxymethyl] aminomethane, 0.25 mM ethylenediaminetetraacetic acid, pH 7.4, aqueous solution). Immediately, using a microcomputer-coupled spectrophotometer (Ultrospec 2000, Pharmacia Biotech Inc.), the absorbance was monitored at 220 nm over 90 s, at 37°C, with intervals of 2 s between consecutive readings. Degradation of H_2O_2 by catalase causes a gradual decrease in the absorbance of the reactive medium at 220 nm, which indicates the catalase activity of the extract. The values of the catalase activity were corrected for the extract protein concentration and the results were expressed as percentage of pulmonary catalase activity relative to group control.

2.11. Pulmonary lipid hydroperoxides

Dosage of lipid hydroperoxides was carried out on methanolic extract of pulmonary tissue as described by Nourooz-Zadeh et al. [29]. A 300-mg portion of the lower pulmonary right lobe was homogenized in 1 ml methanol, using an electric homogenizer (GGS 27, Bosch). After centrifugation (5000×*g*, 5 min, 4°C), a 50-μl aliquot of the supernatant was stored for further measurement of the total proteins, and 90-μl aliquots were disposed into six centrifuge vials (1.5 ml). To three of these vials, 10 μl of methanolic 10 mM triphenylphosphine was added, thereby generating three blanks. To the other three vials, 10 μl of methanol was added. All the six vials were vortexed and then incubated for 30 min at room temperature. After that, 900 μl FOX2 reagent (100 mM xylenol orange, 4 mM BHT, 25 mM sulfuric acid and 250 mM ammonium ferrous sulfate, 90% methanol, 10% ultrapure water) was added to all vials. After mixing, the samples were incubated for another 30 min at room temperature. The absorbance was measured at 560 nm using a spectrophotometer (Ultrospec 2000, Pharmacia Biotech). The results were corrected for the extract protein concentration and expressed as the percentage of the pulmonary concentration of lipid hydroperoxides relative to group control.

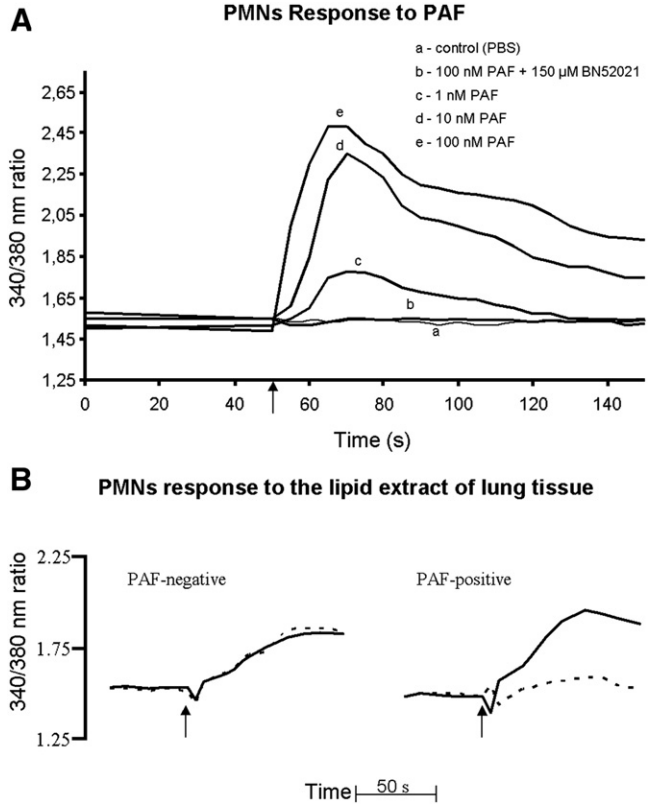

Fig. 1. PAF-promoted Ca^{2+} influx in PMNs obtained from horse blood is shown in A; the arrow indicates the time when 10 µl standard PAF (b–e) or 10 µl PBS (a) was added to PMN suspension; the PAF antagonist, BN52021 (b), was added 4 min before start reading. B, Ca^{2+} influx promoted in PMNs by lipid extract from pulmonary tissue; the left graph is representative for a sample with no PAF bioactivity (from Group C); the right graph is representative for a sample with PAF bioactivity (from Group AS); continuous line represents the response of PMNs in absence of PAF antagonist BN52021; discontinuous line represents the response of PMNs in presence of 150 µM BN52021. Arrow indicates the moment when the lipid extract was added to PMN suspension.

2.12. Isolation of horse polymorphonuclear cells

Horse blood was collected by jugular vein puncture and deposited in a 500-ml blood bag (CPDA-1, JP Pharmaceutical Industry SA, São Paulo, Brazil). Blood was diluted 1:1 (v/v) in PBS, and 40 ml of diluted blood was centrifuged (400×*g*, 40 min, 4°C) over 8 ml Ficoll Paque Plus. After treating the lower phase with hemolytic solution (0.77% NH_4Cl, 0.21% tris[hydroxymethyl]aminomethane, pH 7.4, aqueous solution) for 20 min at 37°C, polymorphonuclear cells (PMNs) were isolated by centrifugation (400×*g*, 6 min, 4°C). The cell pellet was resuspended in 5 ml PBS containing 5 mM glucose. The cellular density was determined in a Neubauer chamber. The cellular viability was about 95%, as determined by trypan blue exclusion. The percentage of neutrophils was over 90% in the PMN suspension, as determined by May-Grünwald/Giemsa staining.

2.13. Measurement of PAF-induced Ca^{2+} influx in PMNs (PAF bioactivity)

The PMNs obtained from horse blood were suspended at the density of 1.0×10^7 cells/ml in PBS containing 5 mM glucose and incubated in the dark with 1 µM Fura-2 AM for 45 min at 37°C. The cells were then washed twice in Ringer solution (142.5 mM NaCl, 4.0 mM KCl, 1.8 mM $CaCl_2$, 1.8 mM $MgCl_2$, 5.0 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 5.0 mM glucose, pH 7.4, aqueous solution) and resuspended in Ringer solution at a density of 2.25×10^6 cells/ml. After incubation for 4 min at 37°C in the dark, 2 ml PMN suspension was disposed in a cuvette and fluorescence was measured at 37°C, with dual excitation at 340 and 380 nm with the emission recorded at 510 nm [30] by a microcomputer-coupled spectrofluorimeter (RF5301PC, Shimadzu, Kyoto, Japan) controlled by the software Super Ion Probe (Shimadzu, Kyoto, Japan). For testing lipid extract of pulmonary tissue for PAF bioactivity, 10 µl of total lipid extract was added to PMN suspension after a baseline reading of 50 s and the response elicited (influx of Ca^{2+}) was monitored for a further 100 s. This procedure was repeated to each sample in the PMN suspension pretreated (4 min before start reading) with 150 µM BN52021 (PAF antagonist). The difference between the maximal responses obtained in presence and in absence of BN52021 (see example in Fig. 1B.), to each sample, was considered as the semiquantitative indicator of PAF bioactivity on lipid extract. This procedure was performed to all groups, and the results of PAF bioactivity were expressed as the 340/380-nm ratio.

Before testing the samples of lipid extracts for PAF bioactivity, each PMN batch was tested with 1, 10 and 100 nM PAF, with or without 150 µM BN52021. The response of horse PMNs to standard PAF is shown in Fig. 1A, which is representative for data obtained in all experiments. Moreover, Fig. 1B shows the graphs representative for one sample positive and for one sample negative for PAF bioactivity.

2.14. Statistical analysis

All values are expressed as the mean±S.E.M. Statistical differences were assessed by ANOVA, followed by Bonferroni's posttest (α=.05).

3. Results

3.1. PAF-induced Ca^{2+} influx in PMNs

In the present work, the whole PAF bioactivity was estimated by a semiquantitative method, which is based on the Ca^{2+} influx elicited by PAF and PAF-like lipids in PMNs. The PAF antagonist BN52021 was used as a negative control in every determination.

The data presented in Fig. 1A are representative for the results obtained in every pretest with authentic PAF. The Ca^{2+} influx was directly proportional to the tested PAF concentrations, and 150 µM BN52021 totally inhibited the PAF-induced calcium influx. Such a high concentration of BN52021 was needed to antagonize high concentrations of PAF. In previous experiments, several different concentrations of BN52021 were tested (results not shown), and 150 µM BN52021 completely inhibited PAF-induced Ca^{2+} influx when the PMNs were exposed to high concentrations of standard PAF (such as 100nM PAF). Moreover, this concentration of BN52021 did not affect Ca^{2+} influx in the presence of calcium ionophore (positive control, results not shown). Each batch of PMN suspension was pretested with different concentrations of authentic PAF, with or without PAF antagonist BN52021, before the estimation of PAF bioactivity in the samples of pulmonary tissue lipid extracts.

The difference between the values of Ca^{2+} influx induced by the lipid extract samples in the presence and in the absence of PAF antagonist BN52021 was used as a semiquantitative indicator of the PAF bioactivity in the pulmonary tissue. In Fig. 1B is shown one representative graph for the PAF bioactivity of Group AS (positive for PAF bioactivity) and one representative for the PAF bioactivity of

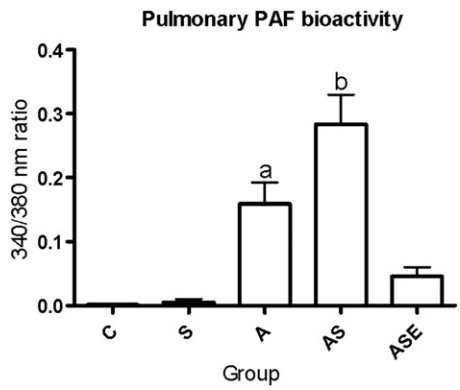

Fig. 2. Pulmonary PAF bioactivity for the following groups: control (C), supplemented with soybean lecithin (S), asthmatic (A), asthmatic supplemented with soybean lecithin (AS) and asthmatic supplemented with soybean lecithin and DL-α-tocopheryl acetate (ASE). The values expressed in this graph are the differences between the maximal responses obtained in the presence and in the absence of the PAF antagonist BN52021, for eight samples of different individuals analyzed in two experiments. Data are presented as mean±S.E.M. [a]$P<.05$ vs. Group C; [b]$P<.05$ vs. Group S, vs. Group A and vs. Group ASE.

Group C (negative for PAF bioactivity). As indicated in this figure, the difference between the maximal responses in the presence and in the absence of BN52021 was clearly higher in Group AS, indicating an elevated lung PAF bioactivity. The results obtained for each group (C, S, A, AS and ASE) were summarized as the mean difference between the maximal responses (topic "PAF bioactivity").

3.2. PAF bioactivity

PAF is an inflammatory mediator involved in several aspects of asthmatic inflammation. Therefore, the pulmonary PAF bioactivity is expected to be increased in asthmatic individuals. Indeed, asthmatic inflammation increased the PAF bioactivity in pulmonary tissue (A=0.159±0.033 340/380-nm ratio vs. C=0.002±0.001 340/380-nm ratio; $P<.05$; Fig. 2). The dietary supplementation with soybean lecithin increased PAF bioactivity significantly (78.0%) only in asthmatic individuals (AS=0.283±0.047 340/380-nm ratio), which suggests an effect of soybean lecithin dependent on the asthmatic inflammation. Dietary supplementation with DL-α-tocopheryl acetate decreased PAF bioactivity by 83.7% (ASE=0.046±0.014 340/380-nm ratio).

3.3. Lipid hydroperoxides

As shown in Fig. 3, pulmonary lipid peroxidation was significantly increased by asthmatic inflammation (Group A, 219.5±26.3%) in comparison to control (100.0±13.4%). In addition, pulmonary lipid peroxidation was 55.9% higher in asthmatic rats receiving dietary supplementation with soybean lecithin (AS=342.1±52.6%) than in nonsupplemented asthmatics (Group A). The asthmatics from Group ASE, which received DL-α-tocopheryl acetate along with soybean lecithin, had a decrease of 98.0% in the pulmonary lipid hydroperoxide concentration compared to asthmatics from AS (ASE=6.9±2.6%; $P<.001$). Interestingly, this results profile is similar to that obtained for PAF bioactivity, which suggests that lipid peroxidation, a process by which PAF-like lipids are generated, can be related to the PAF bioactivity.

3.4. Nitric oxide

Nitric oxide (NO) is one of the reactive species produced in lungs during asthmatic inflammation and plays several roles in oxidative stress. In this work, the production of nitric oxide in lungs was accessed by the BALF concentration of nitrite, a product of in vivo oxidation of nitric oxide. As indicated in Fig. 4, asthmatic inflammation increased NO production in lungs (A=158.6±8.5%) compared to control treatment (C=100.0±5.4%). The dietary supplementation protocol did not affect NO production.

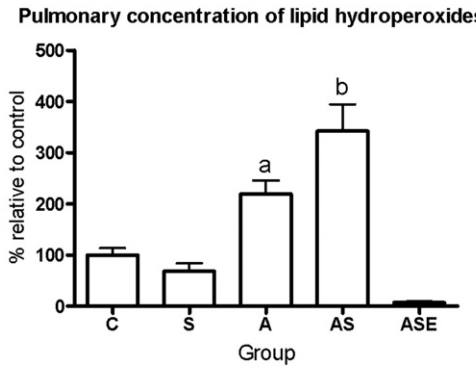

Fig. 3. Pulmonary concentration of lipid hydroperoxides for the following groups: control (C), supplemented with soybean lecithin (S), asthmatic (A), asthmatic supplemented with soybean lecithin (AS) and asthmatic supplemented with soybean lecithin and DL-α-tocopheryl acetate (ASE). Data are presented as mean±S.E.M. of 14 individuals for each group, analyzed in three experiments. [a]$P<.05$ vs. Group C; [b]$P<.001$ vs. Group S, $P<.05$ vs. Group A and $P<.001$ vs. Group ASE.

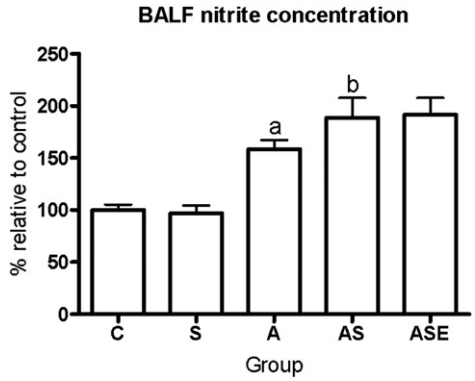

Fig. 4. BALF nitrite concentration for the following groups: control (C), supplemented with soybean lecithin (S), asthmatic (A), asthmatic supplemented with soybean lecithin (AS) and asthmatic supplemented with soybean lecithin and DL-α-tocopheryl acetate (ASE). Data are presented as mean±S.E.M. of 16 individuals for each group, analyzed in three experiments. [a]$P<.05$ vs. Group C; [b]$P<.001$ vs. Group S.

3.5. Catalase

Catalase degrades hydrogen peroxide, preventing the generation of hydroxyl radical by this compound. Therefore, this enzyme plays a central role in the antioxidant system. Pulmonary catalase activity was not affected by asthmatic inflammation (Fig. 5) or by the dietary supplementation protocol.

3.6. Concentration of proteins in BALF

The airway inflammation increases the permeability of the alveolar–capillary barrier, which is responsible for a leakage of plasma proteins to the lumen of airways. The concentration of proteins in BALF was higher in asthmatic (A=187.1±12.0 μg/ml) than in control individuals (C=99.5±15.3 μg/ml; Fig. 6). Moreover, dietary supplementation with soybean lecithin increased this parameter by 42.8% only in asthmatics (AS=267.2±20.7 μg/ml).

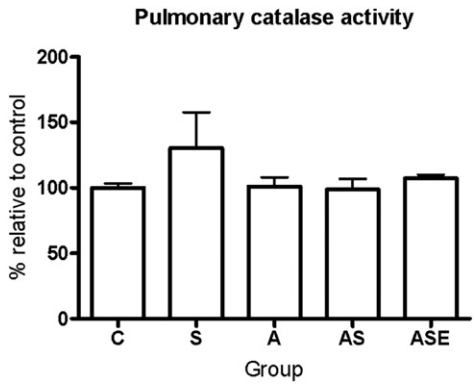

Fig. 5. Pulmonary catalase activity for the following groups: control (C), supplemented with soybean lecithin (S), asthmatic (A), asthmatic supplemented with soybean lecithin (AS) and asthmatic supplemented with soybean lecithin and DL-α-tocopheryl acetate (ASE). Data are presented as mean±S.E.M. of 12 individuals for each group, analyzed in three experiments. There are no statistically significant differences between means.

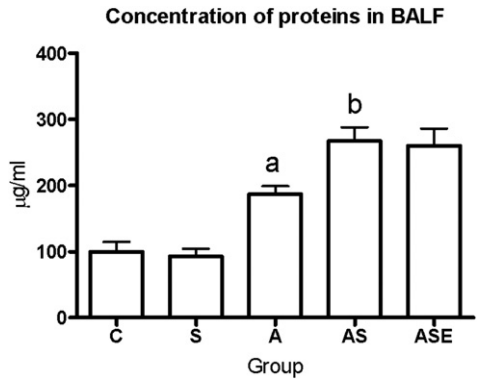

Fig. 6. Concentration of proteins in BALF for the following groups: control (C), supplemented with soybean lecithin (S), asthmatic (A), asthmatic supplemented with soybean lecithin (AS) and asthmatic supplemented with soybean lecithin and DL-α-tocopheryl acetate (ASE). Data are presented as mean±S.E.M. of 16 individuals for each group, analyzed in three experiments. $^{a}P<.05$ vs. Group C; $^{b}P<.001$ vs. Group S, and $P<.05$ vs. Group A.

Treatment with DL-α-tocopheryl acetate did not affect BALF protein concentration.

4. Discussion

This study demonstrates that dietary supplementation with soybean lecithin, a source of PUFA-containing phospholipids, increases pulmonary lipid peroxidation and PAF bioactivity in asthmatics. These effects of dietary soybean lecithin were suppressed by the treatment with DL-α-tocopheryl acetate, suggesting that the generation of PAF-like lipids may be involved in the increase of the PAF bioactivity.

In accordance with the literature [31] and as shown in Fig. 3, pulmonary lipid peroxidation is increased by asthmatic inflammation. Corroborating with previous studies [31,32], this result indicates the occurrence of oxidative stress in lungs of asthmatics. The in vivo lipid peroxidation is initiated when a hydrogen atom, mainly from a PUFA bisallylic system, is abstracted by reactive species such as hydroxyl radical ($OH^{\cdot}$) [33,34]. Thus, due to the presence of the bisallylic system (-CH=CH-CH_2-CH=CH-), PUFAs are prone to peroxidation [35], and diets enriched in PUFA increase the oxidability of biological structures [36,37]. In accordance with these assertions, the present study shows that lipid peroxidation in pulmonary tissue of asthmatics is increased by dietary supplementation with soybean lecithin (Fig. 3). This result is probably due to the high content of PUFAs in soybean lecithin, esterified mainly in phosphatidylcholine.

Lipid peroxidation exerts deleterious effects in asthma, as revised by Wood et al. [16], such as enhancement of airway hyperresponsiveness [31,38], smooth muscle constriction [39,40], airway obstruction, plasma exudation [41] and vascular constriction [42,43]. Therefore, the results above discussed indicate that the lipid pattern of the diet can exert a role in asthma by increasing the lung lipid peroxidation.

One factor that links the lipid peroxidation to its deleterious effects is the nonenzymatic generation of lipid-derived proinflammatory mediators, such as the lipids with PAF bioactivity, named PAF-like lipids [44]. PAF-like lipids are generated by the cleavage of carbon–carbon bonds in the *sn*-2 PUFA residue on phosphatidylcholine molecules, a reaction initiated by lipid peroxidation [18]. As shown in Fig. 2, asthmatic inflammation increased pulmonary PAF bioactivity. Moreover, dietary supplementation with soybean lecithin increased this parameter in lungs of asthmatics. Although the method used does not distinguish between PAF and PAF-like lipids, it is possible, by comparing these results with those from lipid peroxidation, to suppose that the lipid peroxidation-elicited PAF-like generation may be involved in this increase of PAF bioactivity.

In order to verify if the increase of pulmonary PAF bioactivity in asthmatics promoted by soybean lecithin is related to lipid peroxidation, and thus to the generation of PAF-like lipids, the diet of an asthmatic group was supplemented concomitantly with soybean lecithin and DL-α-tocopheryl acetate (Group ASE), a potent inhibitor of lipid peroxidation. As shown in Figs. 2 and 3, both the PAF bioactivity and pulmonary lipid peroxidation were significantly reduced by the treatment with DL-α-tocopheryl acetate, reinforcing the idea that soybean lecithin increased the PAF-like generation. A similar result was obtained by Lehr et al. [45]. These authors exposed hamsters to cigarette smoke, which induces oxidative stress and thus elicits the generation of PAF-like lipids. By treating these animals with vitamin C, an antioxidant involved in regeneration of reduced vitamin E, the in vivo generation of PAF-like lipids was totally inhibited.

Besides the lipid composition, the amount of reactive species and the antioxidant status also influence the extent of lipid peroxidation. The hydroxyl radical, for example, is a highly reactive species capable of removing a hydrogen atom from a bisallylic system, thus initiating the lipid peroxidation process [33]. This radical can be generated by degradation of peroxynitrite [46], which is generated by the reaction between superoxide anion ($O_2^{-}\cdot$) and $NO^{\cdot}$ [47-49]. The production of both $O_2^{-}\cdot$ and $NO^{\cdot}$ occurs simultaneously in activated inflammatory cells [50] and is increased in lungs by asthmatic inflammation [14,15]. Therefore, the $NO^{\cdot}$ contributes to lipid peroxidation [36]. As previously described in the literature [15,51] and shown in Fig. 4, asthmatic inflammation increased the pulmonary production of $NO^{\cdot}$. The supplementation protocol did not alter this parameter.

In addition to $NO^{\cdot}$ and other reactive species, the hydrogen peroxide (H_2O_2) is an important source of $OH^{\cdot}$, which is capable of initiating the lipid peroxidation [52]. In this context, catalase, a component of the enzymatic antioxidant system responsible for degrading H_2O_2, plays a role in preventing lipid peroxidation [53]. As demonstrated here (Fig. 5), and in accordance with literature [54-56], asthmatic inflammation did not alter catalase activity. Also, the supplementation protocol had no effect on this parameter.

Increased production of $NO\cdot$, and even a relatively insufficient catalase activity, could be partially responsible for the increased lipid peroxidation in lungs of asthmatics. However, there were no statistically significant differences in $NO^{\cdot}$ production and catalase activity among the three asthmatic groups; thus, the effect of these two parameters on lipid peroxidation is the same in asthmatics, independently of the supplementation protocol used in this work.

PAF is one of the most potent and versatile proinflammatory mediators and exerts important actions in asthmatic inflammation, such as chemotaxis and activation of leukocytes, vasoconstriction, bronchoconstriction, and bronchial hyperresponsiveness [19,21-23]. In order to evaluate if the alterations in pulmonary PAF bioactivity affect the permeability of the alveolar–capillary barrier, which is an index of the inflammatory status [57], concentration of proteins in BALF was determined. Indeed, asthmatic inflammation significantly increased the concentration of proteins in BALF (Fig. 6), reflecting the effect of the inflammatory process on the integrity of the alveolar–capillary barrier. Moreover, this parameter was significantly increased by dietary supplementation with soybean lecithin, but it was not modified by concomitant supplementation with DL-α-tocopheryl acetate. Thus, the supplementation with soybean lecithin probably alters the permeability of the alveolar–capillary barrier by a way that may involve other proinflammatory mediators than PAF or PAF-like.

In conclusion, dietary supplementation with soybean lecithin increases pulmonary PAF bioactivity, probably by increasing the lipid peroxidation-related generation of PAF-like lipids. Thus, the results presented here can help to explain the mechanism by which some diet-related factors, such as the antioxidant content or the lipid pattern of the diet, affect asthma development.

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