

The Effectiveness of Fish Oil Supplementation in Asthmatic Rats is Limited by an Inefficient Action on ASM Function

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Abstract Episodes of acute exacerbation are the major clinical feature of asthma and therefore represent an important focus for developing novel therapies for this disease. There are many reports that the n-3 fatty acids found in fish oil exert anti-inflammatory effects, but there are few studies of the action of fish oil on airway smooth muscle (ASM) function. In the present investigation, we evaluated the effect of fish oil supplementation on smooth muscle force of contraction in ovalbumin-induced asthmatic Wistar rats, and its consequences on static lung compliance, mucus production, leukocyte chemotaxis and production of proinflammatory cytokines. Fish oil supplementation suppressed the infiltration of inflammatory cells

into the lung in asthmatic animals ($2.04 \pm 0.19 \times 10^6$ cells vs. $3.33 \pm 0.43 \times 10^6$ cells in the control asthmatic group; $P < 0.05$). Static lung compliance increased with fish oil supplementation in asthmatic rats (0.640 ± 0.053 mL/cm H₂O vs. 0.399 ± 0.043 mL/cm H₂O; $P < 0.05$). However, fish oil did not prevent asthma-associated lung eosinophilia and did not affect the concentrations of tumor necrosis factor- α and interleukin-1 β in lung tissue or the proportion of the airways obliterated with mucus. Fish oil had no effect on the force of contraction in asthmatic rats in response to acetylcholine (3.026 ± 0.274 mN vs. 2.813 ± 0.364 mN in the control asthmatic group). In conclusion, although fish oil exerts some benefits in this model of asthma, its effectiveness appears to be limited by an inefficient action on airway smooth muscle function.

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Abbreviations

A	Asthmatic group
Ach	Acetylcholine
AFO	Asthmatic fish oil group
Ai	Airway internal area
Amo	Airway outer muscle area
Ao	Airway outer area
ASM	Airway smooth muscle
BALF	Bronchoalveolar lavage fluid
C	Control group
FO	Fish oil group
IL-1 β	Interleukin 1 β
Iso	Isoproterenol
OVA	Ovalbumin
PBS	Phosphate-buffered saline
Pi	Airway internal perimeter

Pmo	Airway outer muscle perimeter
Po	Airway outer perimeter
PUFA	Polyunsaturated fatty acid(s)
SEM	Standard error of the mean
TNF- α	Tumor necrosis factor- α
W _{Ai}	Inner wall area
W _{Ao}	Adventitial wall area
WAT	Total wall area

Introduction

The anti-inflammatory effects of n-3 polyunsaturated fatty acids (PUFA), found in oily fish and in fish oil supplements, have been extensively studied [1–4]. These effects often involve the incorporation of the n-3 PUFA into cell membranes in order to modulate availability of substrates for syntheses of weak inflammatory eicosanoids and proresolubilization mediators termed resolvins and protectins [1–4]. In addition, recent studies suggest that n-3 PUFA act to prevent activation of the nuclear factor kappa B (NF κ B) signaling pathway, probably by modulating the responsiveness of receptors to inflammatory stimuli, so reducing expression of inflammatory genes [1–4]. As a result of these anti-inflammatory actions fish oil has been used with some success in rheumatoid arthritis, ulcerative colitis, Crohn's disease and psoriasis [2]. However, its effectiveness in asthma has been inconsistent and limited to date [2, 5].

Asthma is an allergic disease characterized by airway hyper-responsiveness, reversible bronchoconstriction, mucus hypersecretion and eosinophilia [6–8]. When in contact with an allergen, the asthmatic patient presents episodes of acute exacerbations, an immediate reaction of bronchoconstriction and concomitant release of proinflammatory mediators. During the occurrence of these episodes, airways obstruction is established as a result of the concentric contraction of smooth muscle, airway wall edema and luminal obstruction with mucus [7]. Acute exacerbation is the major clinical feature of asthma and therefore represents an important focus for the development of novel therapies for this disease. The study of the effect of potential treatments, such as n-3 PUFA, on the contractile properties of airway smooth muscle (ASM) may reveal novel mechanisms of action by which the treatments could interfere with respiratory mechanics.

The hyperresponsiveness and bronchial constriction observed in asthma essentially depend on the ASM functionality [8]. The ASM contractile properties are modulated by inflammatory cytokines, present in high concentrations in asthma. Among them are interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), secreted by leukocytes

and smooth muscle. Both IL-1 β and TNF- α can promote an increase in bronchial responsiveness and in ASM contractility [8–10]. These cytokines promote the recruitment and activation of inflammatory cells, contributing to the perpetuation of inflammatory response [11–13]. IL-1 β induces mucus secretion, T-lymphocyte activation and neutrophil recruitment [9, 12]. TNF- α is a chemoattractant for neutrophils and eosinophils, and induces the expression of adhesion molecules and vascular permeability [11, 13]. Thus, the production of these cytokines is related to the ASM functionality and the inflammatory response.

Despite the on-going study of the antiinflammatory effects of n-3 PUFA, there are few studies of the effect of fish oil on ASM functionality. In the present investigation, we evaluated the effect of fish oil on smooth muscle force of contraction, its consequences on static lung compliance, mucus production, leukocyte chemotaxis and proinflammatory cytokine production. We hypothesized that fish oil supplementation in asthmatic rats has a role in ASM functionality and in related parameters, which could explain, at least in part, the impact of fish oil in asthma.

Materials and Methods

Reagents

TNF- α and IL-1 β ELISA kits were purchased from eBioscience (San Diego, California, USA). Fish oil capsules were donated by Herbarium (Colombo, Paraná, Brazil). Ovalbumin (OVA) (Grade II), aluminium hydroxide (Al(OH)₃), thiopental sodium, MayGrünwald and Giemsa solutions and all other reagents were purchased from Sigma (St. Louis, Missouri, USA).

Study Protocol

Male Wistar rats (aged 8 weeks and weighing 220 $\pm$ 30 g) were purchased from the Biotery of Universidade Federal do Paraná, Curitiba, Brazil. Rats were allowed free access to water and food (52 % by weight of carbohydrates, 21 % by weight of proteins and 4 % by weight of total lipids; Nuvilab CR1, Nuvital Nutrientes Ltda, Curitiba, Paraná, Brazil), and were maintained at a room temperature of approximately 23 °C with 55 % relative humidity and 12 h inverted light/dark cycle. In each experiment, the rats were randomly divided into four groups: control (C), fish oil (FO), asthmatic (A) and asthmatic fish oil (AFO), with four rats in each group. Daily fish oil supplementation was started on day 1 (FO and AFO). Fish oil was administered into the mouth using a micropipette at a daily dose of 1 g/kg of body weight for 21 days. Dose and period of supplementation were based on previous studies in rats in which effects of fish oil were

demonstrated [14–17] and in which n-3 fatty acids incorporation occurred in a short-term period [18], respectively. The body weight of the rats was recorded throughout this period in every 2 days and was not different between the groups (data not shown). On days 5 and 12, allergic sensitization was performed on A and AFO rats by a subcutaneous injection of OVA (1 mg/mL) complexed with 20 mg of $\text{Al}(\text{OH})_3$ adjuvant in phosphate-buffered saline (PBS). C and FO rats received only the adjuvant in PBS. On days 18, 19 and 20 the rats were challenged by exposure to an aerosol of 5 % OVA diluted in sterile PBS using an ultrasonic nebulizer (Ultraneb) for 30 min. Twenty-four hours after the final challenge the animals were given an intraperitoneal injection of thiopental (20 mg/kg), and euthanized by an injection of potassium chloride (2 mmol/kg). The bronchoalveolar lavage fluid (BALF) was collected for enumeration of total leukocytes and leukocyte subsets. After BALF collection the trachea was removed for the isolation of a tracheal ring. Lungs were collected for determination of IL-1 β and TNF- α . Animals that were not used for BALF collection were tracheostomized for the measurement of static lung compliance and after that the lungs were fixed for histological analyses. All procedures were approved by the institutional Animal Ethics Committee (protocol number 468), in accordance with the Brazilian College on Animal Experimentation (COBEA) guidelines.

BALF Collection and Cell Counts

Rats were tracheostomized and BALF was collected by cannulating the upper part of the trachea. The lungs were gently lavaged with 5 mL of PBS. This procedure was repeated 10 times for each animal and 90–95 % of the installed volume was recovered each time. Samples were kept on ice and then centrifuged for 8 min at 290g and 4°C. After centrifugation, pellets were suspended in 500 μL of PBS, for total and differential cell counts. The total number of cells was determined by light microscopy analysis of the cell suspension (at 400 $\times$ magnification) using trypan blue (0.05 %) for dead cell exclusion and a hemocytometer. BALF cells were prepared and stained with May-Grünwald/Giemsa and the slides were subjected to blinded manual differential cell counts in which 200 cells were counted per slide using light microscopy analysis (at 1,000 $\times$ magnification). The percentage of different cell populations was determined and reported as eosinophils, neutrophils, macrophages and lymphocytes.

Determination of the Levels of IL-1 β and TNF- α in Lung Tissues

After BALF collection, the rats were thoracotomized. The lower pulmonary right lobe of the lungs were removed,

washed in PBS and frozen in liquid nitrogen before storage at -80°C for later measurement of the levels of IL-1 β and TNF- α . Lungs were homogenized in PBS (100 mg tissue/mL PBS) and the levels of IL-1 β and TNF- α were determined using commercially available ELISA kits according to the manufacturer's protocols.

Airway Smooth Muscle Responsiveness to Acetylcholine (Ach) or Isoproterenol (Iso)

The experimental model performed with the muscle obtained from the tracheal ring is a classical measurement of the functional alterations in the ASM [19]. The isolated tracheal ring was placed in Normal Ringer solution (NaCl 110 mM, KCl 4 mM, CaCl_2 2 mM, MgCl_2 2 mM, TRIS-MA 10 mM, glucose 1 mM, pH 7.4) pre-gassed with pure O_2 . A single tracheal ring was cut in the medial axis of the cartilage and the ends were fixed using clamps; one end was connected to a MLT0420 Force Transducer (AD Instruments, Bella Vista, Australia) and the other to a movable arm, to adjust the length of the preparation (model 300B; Cambridge Technologies). This preparation was mounted for maximal contraction or relaxation recording in 10 mL tissue baths at 37 °C containing Normal Ringer solution continuously gassed with pure O_2 . Tension was assessed using a Beckman (type RM) chart recorder. The preparation was stretched to 120 % of the slack length. The maximal contraction was obtained by adding acetylcholine (150 μM) to the bath. After the preparation reached the maximal contraction, relaxation was induced by adding isoproterenol (100 μM) into the bath. This protocol was used for all groups studied. Values of force are expressed in mN.

Morphometric Analysis of Airway Wall Dimensions and Inflammation

Lungs were collected and immediately fixed in Karnovsky. Serial sections (3- μm sections taken every 100 μm) of paraffin-embedded lungs were prepared and stained with hematoxylin and eosin. Each section was analysed by a light microscope (Axio-Imager Z2 Zeiss) coupled to a computer and the morphometric measures were taken with the MetaSystem program. All airways that were cut in a cross section in which the short vs. long diameter ratio at the smooth muscle border was >0.6 were examined. The measurements included (1) airway internal perimeter (P_i) and area (A_i); (2) outer muscle perimeter (P_{mo}) and area (A_{mo}); and (3) airway outer perimeter (P_o) and area (A_o), as previously described [20]. Inner wall area (W_{Ai}), adventitial wall area (W_{Ao}), and total wall area (W_{AT}) were calculated as $W_{Ai} = A_{mo} - A_i$, $W_{Ao} = A_o - A_{mo}$, and $W_{AT} = A_o - A_i$. The airway wall thickness

was calculated as WAT/Ai^* , where Ai^* is the ideal area of the lumen of the relaxed airway calculated as $Ai^* = \pi^2/4\pi$, used to normalize airway wall area and minimize differences caused by the size of the bronchioles. The amount of inflammation was assessed by the measurement of the percentage of the mucus-obiterated area from the inner airway area (Ai) of the bronchial sections.

Static Lung Compliance

The animals were tracheostomized for the insertion of a cannula, to the level of the midthorax, coupled to a pressure transducer (AD instruments Bella Vista, Australia). To measure intrapulmonary pressure, the lung of each animal was inflated with 2-mL steps up to 14 mL and the pressure was recorded using an AD/DA converter connected to a PC (power lab system, AD instruments). Static Lung Compliance was determined as $C[st] = \Delta V/\Delta P$, where ΔV is the variance of the volume of air injected in the lung and ΔP is the variance of pressure measured for each animal.

Statistical Analysis

Data are expressed as means $\pm$ SEM. Data were analysed by one-way ANOVA with post-hoc comparisons between groups using Tukey's test, or, when the data were not normally distributed, Kruskal–Wallis test with Dunn's post-hoc test. Analyses were conducted using Prism 4.0 software (GraphPad, San Diego, CA, USA). In all cases differences were considered significant for $P < 0.05$.

Results

Total and Differential Cell Counts in BALF

Table 1 shows the total number of cells and numbers of different cell populations in BALF from all groups. In asthmatic rats (A group) the total number of cells in BALF

was significantly higher than in non-asthmatic controls, indicating a significant infiltration of leukocytes, which is a feature of asthmatic inflammation. Fish oil supplementation suppressed this infiltration of inflammatory cells in asthmatic rats ($P < 0.05$ vs. A group). Infiltrating cells included macrophages, lymphocytes, neutrophils and eosinophils. Asthma resulted in a significantly higher number of eosinophils in BALF, a characteristic of the asthmatic inflammation seen in this model, and this number was even greater after fish oil supplementation ($P < 0.5$ vs. A group). The number of neutrophils was higher in BALF in the A group compared to the non-asthmatic group. However, although neutrophils were numerically lower in the AFO group than in the A group, the difference was not significant ($P > 0.05$), suggesting that fish oil supplementation did not alter the neutrophilic infiltration in the asthmatic lungs. The numbers of macrophages and lymphocytes were not significantly different between the groups, although again numbers of both these cell types were numerically lower in the AFO group than in the A group.

Concentrations of $TNF-\alpha$ and $IL-1\beta$ in Lung Tissue

Lung tissue $TNF-\alpha$ and $IL-1\beta$ were higher in the asthmatic groups compared with non-asthmatic controls and were not altered by fish oil supplementation (Figs. 1, 2).

Airway Smooth Muscle Responsiveness to Acetylcholine (Ach) or Isoproterenol (Iso)

The functional response of the smooth muscle from the tracheal ring to Ach is shown in Fig. 3a. The force of contraction was significantly lower for the smooth muscle from asthmatic rats (2.813 ± 0.364 mN) compared to non-asthmatic controls (5.144 ± 0.273 mN, $P < 0.05$). The force of contraction in the AFO group was also significantly lower (3.026 ± 0.274 mN, $P < 0.05$) when compared to non-asthmatic controls but was not different from that in the

Table 1 Total and differential cell counts in BALF from non-asthmatic and asthmatic rats supplemented or not with fish oil

	Groups			
	C	FO	A	AFO
Total cells ($\times 10^6/\text{mL} \pm \text{SEM}$)	1.29 ± 0.12 $n = 30$	1.74 ± 0.19 $n = 18$	3.33 ± 0.43^a $n = 22$	2.04 ± 0.19^b $n = 17$
Eosinophils ($\times 10^5/\text{mL} \pm \text{SEM}$)	0.07 ± 0.02 $n = 11$	0.11 ± 0.06 $n = 8$	3.37 ± 0.64^a $n = 9$	$6.40 \pm 0.83^{a,b}$ $n = 7$
Macrophages ($\times 10^5/\text{mL} \pm \text{SEM}$)	8.68 ± 1.75 $n = 11$	10.44 ± 1.65 $n = 8$	10.76 ± 1.78 $n = 9$	7.05 ± 1.20 $n = 7$
Lymphocytes ($\times 10^5/\text{mL} \pm \text{SEM}$)	0.57 ± 0.14 $n = 11$	0.50 ± 0.10 $n = 8$	3.78 ± 1.98 $n = 9$	0.57 ± 0.22 $n = 7$
Neutrophils ($\times 10^5/\text{mL} \pm \text{SEM}$)	0.35 ± 0.18 $n = 11$	0.67 ± 0.41 $n = 8$	2.95 ± 1.11^a $n = 9$	0.72 ± 0.27 $n = 7$

^a $P < 0.05$ vs. C

^b $P < 0.05$ vs. A

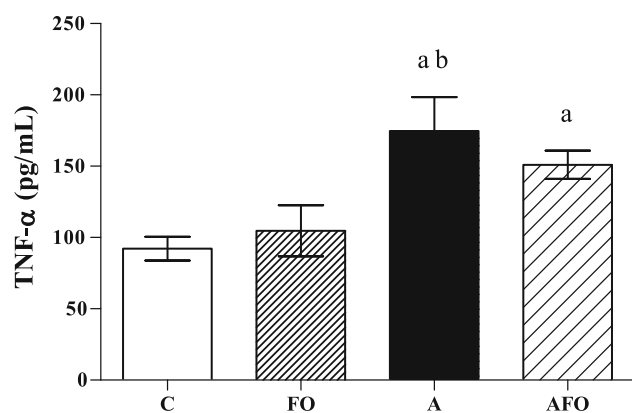


Fig. 1 TNF- α concentrations in lung tissue homogenates obtained from non-asthmatic and asthmatic rats supplemented or not with fish oil. Data are reported as means $\pm$ SEM from C ($n = 30$), FO ($n = 15$), A ($n = 9$) and AFO ($n = 17$) groups. ^a $P < 0.05$ vs. C; ^b $P < 0.05$ vs. FO

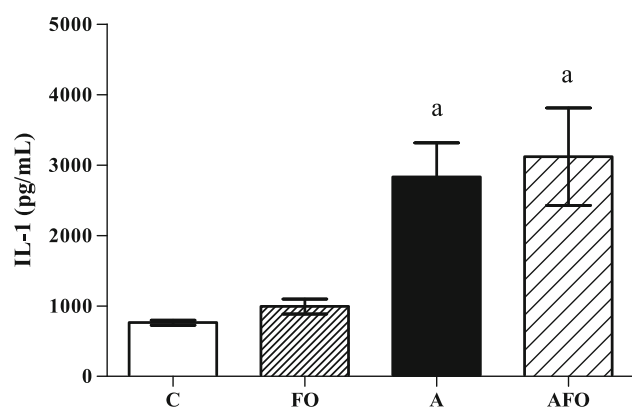


Fig. 2 IL-1 β concentrations in lung tissue homogenates obtained from non-asthmatic and asthmatic rats supplemented or not with fish oil. Data are reported as means $\pm$ SEM from C ($n = 8$), FO ($n = 9$), A ($n = 11$) and AFO ($n = 12$) groups. ^a $P < 0.05$ vs. C

A group, suggesting that fish oil supplementation has little action on airway smooth muscle function in asthmatic rats. However, the airway smooth muscle response to Ach in the non-asthmatic FO group was also significantly lower (3.120 ± 0.215 mN, $P < 0.05$) than in the controls, which shows that fish oil supplementation has direct effects on smooth muscle function in the absence of asthma. Figure 3b shows the functional response of the smooth muscle from the tracheal ring to Iso, a β_2 -adrenergic receptor agonist. The relaxation of the smooth muscle was significantly higher in asthmatic rats (0.707 ± 0.029 mN) compared to non-asthmatic controls (0.522 ± 0.051 mN, $P < 0.05$). Fish oil supplementation in asthmatic rats significantly reduced (0.364 ± 0.032 mN) the relaxation of the smooth muscle compared to the asthmatic group.

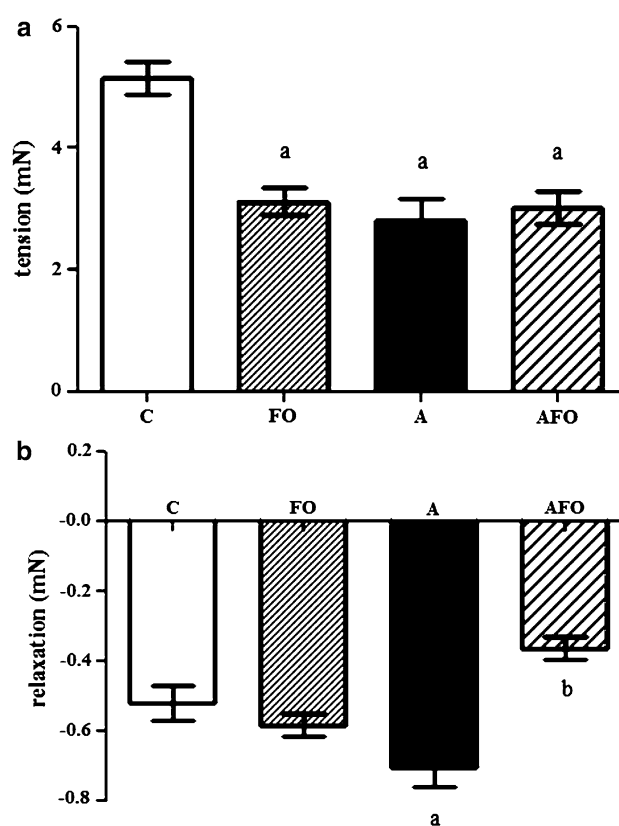


Fig. 3 Force of contraction (a) and relaxation (b) of airway smooth muscle measured in response to 150 μ M of Ach and to 100 μ M of Iso, respectively. **a** Data are means $\pm$ SEM from C ($n = 8$), FO ($n = 6$), A ($n = 8$) and AFO ($n = 8$) groups; ^a $P < 0.05$ vs. C. **b** Data are means $\pm$ SEM from C ($n = 12$), FO ($n = 8$), A ($n = 8$) and AFO ($n = 4$) groups; ^a $P < 0.05$ vs. C; ^b $P < 0.05$ vs. A

Morphometric Analysis of Airway Wall Dimensions and Inflammation

Table 2 summarizes data from morphometric analysis. There were no significant differences in number and size of bronchioles among the four experimental groups, indicating no significant biases in terms of airways selection. Normalized airway wall dimensions were not different between the groups studied, indicating that the thickness of the smooth muscle layer or the adventitial layer were not altered by either asthma or fish oil. The area that was obliterated with mucus was determined as percentage of the internal area of the bronchioles. The mean from the obliterated area was significantly higher in the A and AFO groups when compared to the C group. Fish oil supplementation did not alter the area of mucus, as the means were not different between the FO and C groups or between the AFO and A groups. The average percentage of bronchioles that presented mucus was 50 % from C, 46.2 % from FO, 54.5 % from A, and 70 % from AFO.

Table 2 Morphometric data obtained from histological analysis of lung tissue from non-asthmatic and asthmatic rats supplemented or not with fish oil

	Groups			
	C	FO	A	AFO
Total number of bronchioles	14	13	11	10
Number of bronchioles obtained from individual animals	2.8 ± 0.4	3.3 ± 0.6	2.8 ± 0.5	3.3 ± 0.9
Pi (μm ± SEM)	551.1 ± 111.2	411.0 ± 56.8	391.6 ± 45.1	500.0 ± 74.8
W _{Ai} (μm ² ± SEM)	20,621 ± 4,657	18,685 ± 2,831	15,973 ± 2,502	25,680 ± 6,068
W _{Ao} (μm ² ± SEM)	12,314 ± 3,186	9,446 ± 1,542	13,473 ± 2,701	17,340 ± 4,411
W _{AT} (μm ² ± SEM)	32,936 ± 7,783	28,138 ± 4,266	29,445 ± 4,785	43,020 ± 10,345
W _{AT} /A _i * (μm ² /μm ± SEM)	0.21 ± 0.05	0.30 ± 0.06	0.26 ± 0.04	0.22 ± 0.03
W _{Ai} /A _i * (μm ² /μm ± SEM)	0.13 ± 0.03	0.20 ± 0.04	0.14 ± 0.02	0.13 ± 0.01
Number of bronchioles with mucus	7	6	6	7
Mucus (% of obliterated area from A _i ± SEM)	8.95 ± 1.87	16.15 ± 6.00	36.67 ± 8.51 ^a	33.25 ± 4.29 ^a

Data are reported as means ± SEM from C (*n* = 5), FO (*n* = 4), A (*n* = 4) and AFO (*n* = 3) groups

^a *P* < 0.05 vs. C

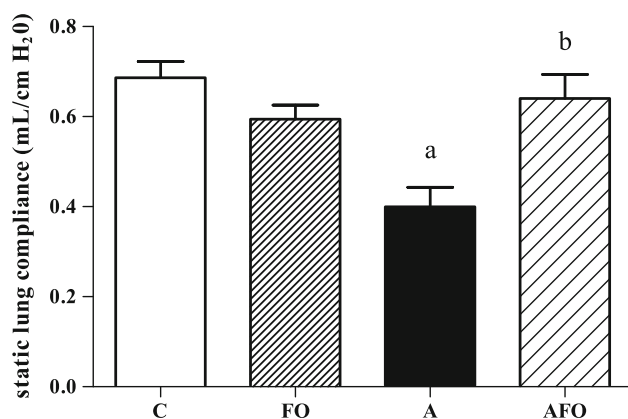


Fig. 4 Static lung compliance in non-asthmatic and asthmatic rats supplemented or not with fish oil. Data are reported as means ± SEM from C (*n* = 12), FO (*n* = 11), A (*n* = 9) and AFO (*n* = 8) groups; ^a*P* < 0.05 vs. C and FO; ^b*P* < 0.05 vs. A

Static Lung Compliance

Figure 4 shows the static lung compliance. Lung compliance in asthmatic rats was significantly lower than in controls. Fish oil supplementation resulted in higher lung compliance than in the A group and this was not different from that seen in the non-asthmatic control group.

Discussion

In the present study, we observed that the supplementation of asthmatic rats with fish oil was able to reduce leukocyte numbers in BALF, probably as a result of decreased chemotaxis, supporting an antiinflammatory role of this supplement. However, fish oil supplementation promoted an

increase in eosinophil infiltration to the lungs in asthmatic rats. This may be a deleterious effect since eosinophils play an important role in maintaining inflammation [21]. Various chemokines have been described as critical factors involved in the recruitment of particular subsets of inflammatory cells from the circulation into the lungs via interaction with chemokine receptors [22]. The cytokines TNF-α and IL-1β are involved in the recruitment and activation of neutrophils and eosinophils [11–13]. However, the concentration of these cytokines in the lung remained high with fish oil supplementation of asthmatic rats. Therefore the reduced chemotaxis and resulting leukocytic infiltration of the lung, seems not to involve a modification of production of these two cytokines, which were not different between fish oil treated asthmatic rats and asthmatic controls. The n-3 PUFA in fish oil could reduce the expression of receptors for chemoattractants [23], so decreasing neutrophil responsiveness to the presence of chemoattractants, or modify the expression of adhesion molecules [23] or alter the profile of lipid mediators which are involved in neutrophil movement [24]. Since leukocyte migration does not depend on a single chemokine [25], the effect of fish oil could occur through several simultaneous actions of the different components of adhesion and migration. Furthermore, eosinophil accumulation is a combination of both increased migration and prolonged survival, which is under the influence of locally generated growth factors, and is considered to be an important mechanism for selective eosinophil accumulation in allergic disease [26]. Amongst the factors involved in prolonging eosinophil survival is granulocyte-macrophage colony-stimulating factor (GM-CSF) [27]. Eosinophil migration in asthma may also be regulated by chemokines secreted by the bronchial epithelium, such as

RANTES, eotaxin, interleukin-4 (IL-4) and interleukin-5 (IL-5), or by increasing adhesion receptors present in epithelial, such as ICAM-1 [27]. It is possible that n-3 PUFA affect one (or more) of these factors related to eosinophil migration, adhesion and activation [18].

Eosinophilia is a marker of allergic reactions [28] and eosinophils are involved in airway hyperresponsiveness [28] and episodes of acute exacerbation [21]. Eosinophils contain charged proteins such as major basic protein, eosinophil cationic protein, and eosinophil peroxidase which affect muscarinic receptors, resulting in increased responsiveness of ASM [29, 30]. Thus, the lack of effect of supplemental fish oil on the functional responses of ASM in asthmatic rats may, at least in part, relate to eosinophil infiltration. Asthmatic inflammation per se altered the force of contraction in the ASM, an effect which could be linked to the increased levels of TNF- α and IL-1 β as well as the infiltration of eosinophils in the lung. There is existing evidence that these factors in combination are able to modify ASM contraction [8–10, 31]. The lower force of contraction in the ASM from asthmatic rats can be explained by the removal of the ASM from the inflammatory environment: as the smooth muscle was pre-contracted in this environment it may have responded poorly when challenged with Ach. In the control group, where inflammation was absent, the smooth muscle could exhibit a normal force of contraction. The direct effect of fish oil on the ASM response to Ach might be due to the modification of transduction factors [32] and/or to fatty acid incorporation into the smooth muscle [33]. Jo and coworkers [34] demonstrated that the fatty acids present in fish oil are incorporated into the membrane of the airways muscle cells, and consequently act to reduce mRNA levels of voltage-dependent sodium channels. Since there is no evidence of structural changes indicative of airway wall thickening in this model, effects of asthma and of fish oil on the force of contraction of ASM seem to be important observations.

Although fish oil supplementation did not result in a difference in the force of contraction of ASM in asthmatic rats, there was a reduction in relaxation in response to Iso. This indicates that n-3 PUFA act on the contraction and relaxation of smooth muscle by different mechanisms. In asthma, ASM hyperresponsiveness is more strongly determined by the increased stimuli that promote muscle contraction than by the stimuli that result in relaxation [19, 29, 30, 35–37]. Altogether, these results indicate that fish oil supplementation in asthmatic rats has only limited action on ASM function. The reduction of muscle relaxation in response to Iso promoted by fish oil could interfere with clinical treatment in asthma attacks, since Iso is used for immediate relaxation of airways and release of airflow [36]. Thus, supplementation of asthmatic subjects with fish

oil could reduce the response to this bronchodilator, having an adverse effect on the control of asthma attacks. However, clinical investigations are necessary to investigate these effects further.

The literature shows that an increase in the tension of the ASM reduces the diameter of the airways [38–41], which is one of the factors that leads to decreased lung compliance [42]. Indeed, the asthmatic animals supplemented with fish oil exhibited higher static lung compliance, accompanied by decreased basal tension of the ASM and the same relation was observed in asthmatic animals. Although the presence of mucus in the airways affects the static compliance [43], in this study this relation was not seen. Dietary supplementation with fish oil in asthmatic animals was ineffective in reducing the amount of mucus present. As the amount of mucus and inflammatory cytokines were not altered by fish oil supplementation in asthmatic animals, it is possible that the basal tension of the muscle is the main factor that affects the lung compliance.

The current study identified that fish oil given at a dose of 1 g/kg body weight each day for 21 days affected some inflammatory responses (leukocyte infiltration into the lungs) but not others (BALF eosinophilia, TNF- α and IL-1 β) and improved some inflammation-related airways phenomena (compliance) but not others (obliteration and ASM force of contraction). These differential effects may be due to the dose of n-3 PUFA being given or to the duration of the supplementation being short. The latter may be particularly important for smooth muscle which may take longer than 21 days to accumulate sufficient n-3 PUFA to influence its function. It is important to note that the daily dose of 1 g/kg body weight is high compared with doses used in human studies [2, 5].

In summary, fish oil supplementation had an anti-inflammatory effect in asthmatic rats, since it reduced leukocyte infiltration into the lungs, and it improved lung compliance in these animals. However, other aspects of inflammation such as eosinophilia and two inflammatory cytokines in BALF, and inflammation-related phenomena such as airway obliteration and ASM force of contraction were not affected by fish oil in asthmatic rats. The latter is the main feature that promotes asthma exacerbation. In conclusion, the effectiveness of fish oil supplementation in asthmatic rats may be limited by an inefficient action on parameters associated to ASM function, which may relate either to an incomplete reduction of inflammation or to a resistance of ASM to the effects of n-3 PUFA. Such a lack of effect may be due to the dose of n-3 PUFA being given or to the short-term supplementation.

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Conflict of interest The authors declare that they have no conflict of interest.

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