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Variations in Inflammatory Cells and IL-6 in Long-Distance Runners Susceptible to Exercise-Induced Bronchospasm and Previously Treated with Salbutamol

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Abstract

Background: Exercise-Induced Bronchospasm (EIB) is an inflammatory condition characterized by severe airway constriction following the mobilization of inflammatory cells and interleukin-6 (IL-6). When severe, EIB can require the use of pressurized salbutamol to treat athletes. This study investigated the nature of the systemic changes in inflammatory cells and post-exercise IL-6 concentrations after salbutamol treatment in EIB-susceptible distance runners. **Materials and Methods:** This was an experimental study that enrolled 12 long-distance runners. In Session A, the participants completed a treadmill exercise test, and those who had a maximum expiratory volume per second (FEV1) that was decreased by at least 10% compared to their base value were placed in the EIB-susceptible group (EIB+) (n = 6). Those whose FEV1 did not meet this criterion were placed in the nonresponsive (EIB−) group (n = 6). Before the Session B exercise, athletes in the BIE+ group inhaled two puffs of salbutamol (EIB+ Salb), while their EIB− counterparts received no treatment. Spirometry was performed before and after the exercise using a Spirobank G portable spirometer. Blood samples were taken before, immediately after and 2 hours after the stress test. **Results:** The mean post-exercise FEV1 values were not significantly different ($p > 0.05$) between the EIB+ Salb group and the EIB− group. The systemic changes in inflammatory cells and IL-6 concentrations in the EIB+ runners after salbutamol treatment were similar to those observed in their EIB− counterparts. **Conclusion:** Salbutamol pretreatment improved the systemic immune status of EIB-susceptible athletes.

Keywords

Exercise-Induced Bronchospasm, Salbutamol, Inflammatory Cells, Interleukin-6

1. Introduction

Exercise-Induced Bronchospasm (EIB) is an inflammatory condition characterized by an exaggerated constriction of the pulmonary bronchi. During BIE, activation of inflammatory cells has been observed, in particular neutrophils, eosinophils and mast cells [1] [2] [3]. In addition, endurance exercise induces a post-exercise increase in Interleukin-6 (IL-6) [4] [5].

It requires pharmacological management in sensitive athletes through the use of a short-acting β2-agonist such as salbutamol. Inhalation of 2 puffs of salbutamol before exercise inhibits the symptoms of EIB by stimulating β2 receptors on immune cells (such as mast cells) and acting as an antagonist of inflammatory mediators by inhibiting their release thereby inducing smooth muscle relaxation in the airways [6]. Salbutamol inhaled before exercise can attenuate EIB in 80% - 95% of patients and its effectiveness lasts for 2 - 3 hours of exercise [7]. Pillard *et al.* [8] suggested that salbutamol can prevent and treat EIB symptoms in athletes, with or without a diagnosis of asthma, by relieving asthma symptoms. Jackson *et al.* [9] showed that treating EIB-sensitive soccer players with salbutamol and other inhalants improved airway inflammation and reduced airway hyperresponsiveness.

In an animal model, Romberger *et al.* [10] demonstrated that salbutamol pretreatment reduced the influx of neutrophils into the airways and the release of IL-6 and TNF-α into the Broncho alveolar lavage fluid following a single exposure to organic dust. The authors also showed that salbutamol has anti-inflammatory effects. Another study showed that after the induction of inflammation in Wistar rats, leukocyte accumulation and exudate volume were significantly inhibited by salbutamol administration [11]. The authors also found that salbutamol decreased IL-1β production.

Assuming that inflammatory cells and IL-6 are mobilized by the onset of bronchospasm, the administration of salbutamol in its reversible function should restore the initial level of inflammatory cells and IL-6. The present study investigated systemic changes in inflammatory cells and IL-6 after salbutamol treatment in EIB-sensitive distance runners.

2. Materials and Methods

2.1. Study Participants

Twelve male distance runners participated in this study. They were recruited from various athletic clubs in Brazzaville. After the diagnosis of BIE, the athletes were divided into two groups: one group was made up of runners sensitive to BIE

and then treated with salbutamol (BIE+ Salb) (n = 6), and another group was made up of runners not sensitive to BIE (EIB−) (n = 6). Aged 23 on average, these athletes participate in national athletics championships, international competitions and the international half-marathon in Brazzaville, Republic of Congo.

2.2. Procedures

Athletes were previously subjected to a treadmill stress test and a spirometric test to diagnose subjects susceptible to BIE (BIE+). Before treatment with Salbutamol, the BIE+ were subjected to blood samples before exercise, after and 2 hours after exercise in order to evaluate the variations of inflammatory cells and IL-6. One week later, the BIE+ and BIE− subjects were also subjected to the same exercise test, a spirometry test before and after exercise, blood samples before exercise, after and 2 hours after exercise. Salbutamol treatment was administered only to subjects in the BIE+ group (BIE+ Salb) before exercise testing. The tests were carried out in the weight room of the National Football Training Center at the MASSAMBA Débat stadium in Brazzaville and took place from 7:30 am to 10:30 am. The participants were informed to abstain from any sports practice 48 hours before the tests and from any consumption of alcohol, coffee or drugs on the day of the tests. Blood samples were collected by health workers using standard venipuncture techniques.

2.3. Spirometry Test

Spirometry was performed before and 5 minutes after the stress test using a Spirobank G portable spirometer (Medical International Research). This device made it possible to measure the pulmonary function parameters.

2.4. Administration of Salbutamol

Salbutamol is administered 10 - 15 minutes before exercise to prevent symptoms of EIB and exercise-induced asthma (EIA) for up to 4 hours [8]. According to the clinical recommendations of the Global Initiative of Asthma (GINA) [12] and the clinical guidelines of the American Thoracic Society (ATS) [13], beta-agonists or short-acting adrenergic agent such as salbutamol can be prescribed for sport to prevent EIB, if taken immediately before exercise.

In this study, salbutamol was administered 15 minutes before exercise to subjects who were sensitive to EIB (EIB+). The dose administered or inhaled was 200 µg, *i.e.*, two puffs of 100 µg/puff salbutamol.

2.5. Stress Test

In the stress test, each athlete continuously ran on a treadmill whose speed was increased every 3 minutes by 1.5 $km \cdot h^{-1}$ from 7.5 $km \cdot h^{-1}$ until exhaustion. Exhaustion was evidenced by the subject's inability to maintain treadmill speed.

2.6. Collection of Blood Samples

In the EIB+ group, salbutamol was administered 15 minutes before the exercise

test. However, blood samples were taken 10 minutes after inhaling two puffs of salbutamol. In the EIB− group, blood samples were taken before exercise at the same time as their EIB+ Salb colleagues.

Blood samples were also taken immediately after and 2 hours after the stress test in both the sensitive group who were pre-treated with salbutamol (EIB+ Salb) and the non-sensitive group (EIB−).

2.7. Immunological Laboratory Tests

2.7.1. Examination of Blood Count or Complete Blood Count

Blood count examinations were carried out using an Elite 3 automaton (China) from blood samples taken before and after exercise. The concentrations of leukocytes, lymphocytes, monocytes and granulocytes (neutrophils, eosinophils and basophils) were determined.

2.7.2. ELISA

The plasma IL-6 levels were determined using an IL-6 ELISA kit (human) according to the manufacturer's protocol (Aviva Systems Biology, Corporation, San Diego, USA). The ELISA was carried out at the Louis Pasteur National Laboratory in Brazzaville.

2.8. Variables Studied

The status of each subject in relation to their sensitivity to EIB (EIB− or EIB+ Salb) and the time of the measurements (pre-exertion and post-exertion) were independent variables. Inflammatory cell and IL-6 concentrations were dependent variables.

2.9. Statistical Analysis

Before treatment with salbutamol, the mean values of concentrations of inflammatory cells and those of IL-6 observed before exercise, immediately after and 2 hours after exercise were compared by the Friedman test in BIE+ subjects.

The descriptive statistics generated were the means and standard deviations of the total group, the group susceptible to bronchospasm and pretreated with salbutamol (EIB+ Salb), and the group that was not susceptible to bronchospasm (EIB−). Data normality and the homogeneity of the variances were verified by the Kolmogorov-Smirnov test and the Fischer-Snedecor F test, respectively. The mean values of the anthropometric characteristics, forced expiratory volume in one second (FEV1), distances traveled and times achieved were compared using the Mann-Whitney test. The Mann-Whitney test was also used assess any differences between the EIB+ Salb group and the EIB− group in the mean concentrations of inflammatory cells and IL-6 recorded before exercise.

The mean concentrations of inflammatory cells and IL-6 observed before exercise, immediately after, and 2 hours after exercise were compared by the Friedman test within the EIB+ Salb and EIB− groups. When the Friedman test was significant, a binary comparison of the immunological variables was carried out

using the Wilcoxon test.

Moreover, when the Friedman test is not significant, the binary comparison of immunological variables by the Wilcoxon test is not possible.

Variable data were recorded and processed using Stat View 5 (version 5) from Abacus Concepts Inc. (Berkeley, CA, USA). The significance level was set at $p < 0.05$.

2.10. Ethical Considerations

This study was approved by the Scientific Council of the Higher Institute of Physical and Sports Education of Marien Ngouabi University in the Republic of Congo in accordance with the 1975 Helsinki Declaration relating to ethics.

3. Results

The mean values of the concentrations of the leukocyte subsets and of IL-6 obtained before exercise, after and 2 hours after the exercise test in BIE+ subjects are presented in Table 1. It appears that the concentrations granulocytes, neutrophils and basophils increased significantly over time up to 2 hours after exercise. A significant difference in the mean values of plasma IL-6 concentrations recorded before exercise, immediately after and 2 hours after exercise was observed in BIE+ before treatment with salbutmol (Table 1).

Table 1. Effects of exercise on leukocyte subset and plasma IL-6 responses in BIE+ distance runners (n = 6) before salbutamol treatment.

	Before effort	After effort	2 h after effort	T
	Mean ± SD	Mean ± SD	Mean ± SD	p-value
Leukocytes (10^3/μL)				
BIE+	4.593 ± 2.280	5.662 ± 1.967	5.648 ± 1.998	0.115
Lymphocytes (10^3/μL)				
BIE+	2.403 ± 1.374	3.265 ± 1.203	2.502 ± 1.336	0.846
Monocytes (10^3/μL)				
BIE+	0.615 ± 0.470	0.618 ± 0.428	0.693 ± 0.413	0.260
Granulocytes (10^3/μL)				
BIE+	1.575 ± 0.561	1.781 ± 0.641	2.455 ± 1.181	0.009
Neutrophils (10^3/μL)				
BIE+	1.559 ± 0.556	1.764 ± 0.636	2.431 ± 1.170	0.009
Eosinophils (10^3/μL)				
BIE+	0.011 ± 0.004	0.013 ± 0.005	0.017 ± 0.008	0.009
Basophils (10^3/μL)				
BIE+	0.0047 ± 0.0017	0.0053 ± 0.0019	0.0074 ± 0.0035	0.009
IL-6 (pg/ml)				
BIE+	0.1078 ± 0.0149	0.1578 ± 0.0185	0.1168 ± 00063	0.009

EIB+: subjects' susceptible to exercise-induced bronchospasm; T: friedman test.

The anthropometric characteristics and respiratory parameters (age, height, weight, body mass index (BMI), and post-exercise FEV1) did not significantly differ between the EIB+ Salb group and the EIB− group ($p > 0.05$) (Table 2).

The immune parameters recorded before exercise in the EIB− and EIB+ Salb athletes are compared and summarized in Table 3. The concentrations of inflammatory cells and IL-6 recorded before exercise were not significantly different in the EIB− and EIB+ Salb groups (Table 3).

Table 2. Comparison of the anthropometric characteristics and post-exercise FEV1 values in the EIB− and EIB+ Salb groups.

	Total group (n = 12)			EIB− (n = 6)			EIB+ Salb (n = 6)			T
	Mean	±	SD	Mean	±	SD	Mean	±	SD	p-value
Age (year)	23.00	±	3.643	22.333	±	4.227	23.667	±	3.204	0.871
Height (cm)	169.833	±	5.750	170.333	±	4.274	169.333	±	7.339	0.872
weight (kg)	59.750	±	6.032	61.333	±	5.391	58.167	±	6.706	0.294
BMI (kg/m^2)	20.707	±	1.691	21.102	±	0.915	20.312	±	2.254	0.631
FEV1 post (L)	3.410	±	0.488	3.685	±	0.362	3.135	±	0.459	0.078

BMI: Body Mass Index; FEV1 post: Forced Expiratory Volume in one second after exertion; T: comparison test of mean values between the EIB− group and the EIB+ Salb group.

Table 3. Comparison of the mean pre-exercise inflammatory cell and IL-6 concentrations in the EIB− and EIB+ Salb groups.

	Before exercise						T
	EIB− (n = 6)			EIB+ Salb (n = 6)			
	Mean	±	SD	Mean	±	SD	p-value
Leukocytes ($10^3/\mu L$)	4.077	±	1.065	4.103	±	1.047	0.873
Lymphocytes ($10^3/\mu L$)	1.837	±	0.565	1.787	±	0.398	0.522
Monocytes ($10^3/\mu L$)	0.488	±	0.369	0.410	±	0.095	0.469
Granulocytes ($10^3/\mu L$)	1.747	±	0.601	1.903	±	1.148	0.873
Neutrophils ($10^3/\mu L$)	1.729	±	0.595	1.884	±	1.137	0.873
Eosinophils ($10^3/\mu L$)	0.012	±	0.004	0.013	±	0.008	0.872
Basophils ($10^3/\mu L$)	0.005	±	0.002	0.006	±	0.003	0.871
IL-6 (pg/ml)	0.069	±	0.016	0.079	±	0.029	0.423

EIB−: group of subjects not susceptible to exercise-induced bronchospasm; EIB+ Salb: group of subjects sensitive to exercise-induced bronchospasm and pre-treated with salbutamol; T: Mann-Whitney test.

The mean concentrations of leukocyte subsets and IL-6 obtained before exercise, after exercise, and 2 hours after exercise in the EIB+ Salb and EIB− groups are presented in Table 4. The total number of leukocytes increased significantly after the physical effort and returned to the initial value by 2 hours after the effort in both groups. However, lymphocytosis that occurred immediately after exercise but returned to baseline 2 hours after exercise was also observed in both groups. The concentrations of granulocytes, neutrophils and basophils increased significantly over time up to 2 hours after exercise in both groups. A significant difference in monocytes and eosinophils was observed in the EIB− group (Table 4). The mean concentrations of plasma IL-6 obtained before exercise, immediately after exercise and 2 hours after exercise were not significantly different ($p > 0.05$) between the two groups (Table 4).

Table 4. Effect of exercise on leukocyte subset and plasma IL-6 responses in distance runners in the EIB− and EIB+ Salb groups (n = 6 each).

	Before exercise			After exercise			2 h after exercise			T
	Mean	±	SD	Mean	±	SD	Mean	±	SD	p-value
Leukocytes (10^3/µL)										
EIB−	4.077	±	1.065	5.965	±	1.266	4.852	±	1.088	0.006
EIB+ Salb	4.103	±	1.047	6.460	±	1.722	4.425	±	1.120	0.042
Lymphocytes (10^3/µL)										
EIB−	1.837	±	0.565	3.343	±	0.846	1.690	±	0.610	0.009
EIB+ Salb	1.787	±	0.398	3.722	±	1.270	1.428	±	0.471	0.006
Monocytes (10^3/µL)										
EIB−	0.488	±	0.369	0.535	±	0.218	0.288	±	0.168	0.023
EIB+ Salb	0.410	±	0.095	0.453	±	0.206	0.323	±	0.099	0.130
Granulocytes (10^3/µL)										
EIB−	1.747	±	0.601	2.085	±	0.848	2.870	±	0.962	0.015
EIB+ Salb	1.903	±	1.148	2.285	±	1.103	2.675	±	1.085	0.016
Neutrophils (10^3/µL)										
EIB−	1.729	±	0.595	2.064	±	0.848	2.841	±	0.953	0.016
EIB+ Salb	1.884	±	1.137	2.262	±	1.103	2.648	±	1.074	0.015
Eosinophils (10^3/µL)										
EIB−	0.012	±	0.004	0.015	±	0.006	0.020	±	0.007	0.015
EIB+ Salb	0.013	±	0.008	0.016	±	0.008	0.018	±	0.008	0.070
Basophils (10^3/µL)										
EIB−	0.005	±	0.002	0.006	±	0.003	0.009	±	0.003	0.015
EIB+ Salb	0.006	±	0.003	0.007	±	0.003	0.008	±	0.003	0.016
IL−6 (pg/ml)										
EIB−	0.069	±	0.016	0.063	±	0.007	0.069	±	0.007	0.513
EIB+ Salb	0.079	±	0.029	0.068	±	0.009	0.071	±	0.008	0.846

EIB−: group not susceptible to exercise-induced bronchospasm; EIB+ Salb: group susceptible to exercise-induced bronchospasm and pretreated with salbutamol; T: friedman test.

Compared to the modalities (before effort, after effort and 2 hours after effort) concerning the concentrations of leukocytes, lymphocytes, monocytes, granulocytes, neutrophils, eosinophils, basophils and IL-6, the Friedman test carried out showed the differences significant leukocytes, lymphocytes, monocytes, granulocytes, neutrophils, eosinophils and basophils in BIE− and leukocytes, lymphocytes, granulocytes, neutrophils and basophils in BIE+. The binary tests (Wilcoxon) concerned the concentrations for which the Friedman test was significant.

To examine the variation in blood leukocyte subsets concentrations, we compared the mean concentrations recorded before and after exercise in the EIB− and EIB+ Salb groups (Table 5(a)). The mean leukocyte and lymphocyte concentrations before and immediately after exercise were significantly different (Table 5(a)).

Table 5. (a) Comparison of leukocyte subsets concentrations before and immediately after exercise in the EIB− and EIB+ Salb groups (n = 6 each); (b) Comparison leukocyte subsets concentrations before and 2 hours after exercise in the EIB− and BIE+ Salb groups (n = 6 each).

(a)

	Before exercise			After exercise			T	Delta
	Mean	±	SD	Mean	±	SD	p-value	%
Leukocytes (10^3/µL)								
EIB−	4.077	±	1.065	5.965	±	1.266*	0.028	46.30
EIB+ Salb	4.103	±	1.047	6.460	±	1.722*	0.046	57.44
Lymphocytes (10^3/µL)								
EIB−	1.837	±	0.565	3.343	±	0.846*	0.028	81.98
EIB+ Salb	1.787	±	0.398	3.722	±	1.270*	0.029	10.28
Monocytes (10^3/µL)								
EIB−	0.488	±	0.369	0.535	±	0.218	0.500	9.63
Granulocytes (10^3/µL)	.							
EIB−	1.747	±	0.601	2.085	±	0.848	0.075	1.34
EIB+ Salb	1.903	±	1.148	2.285	±	1.103	0.076	2.07
Neutrophils (10^3/µL)								
EIB−	1.729	±	0.595	2.064	±	0.848	0.075	1.37
EIB+ Salb	1.884	±	1.137	2.262	±	1.103	0.074	2.06
Eosinophils (10^3/µL)								
EIB−	0.012	±	0.004	0.015	±	0.006	0.076	25.00
Basophils (10^3/µL)						.		
EIB−	0.005	±	0.002	0.006	±	0.003	0.074	20.00
EIB+ Salb	0.006	±	0.003	0.007	±	0.003	0.075	16.66

EIB−: group not susceptible to exercise-induced bronchospasm; EIB+ Salb: group susceptible to exercise-induced bronchospasm pre-treated with salbutamol; T: Wilcoxon test; Delta %: variation in percentage of the values recorded immediately after exercise compared to the values obtained before exercise in the subjects of the EIB− group and those of the EIB+ Salb group. *: $p < 0.05$; SD: Standard Deviation.

(b)

	Before exercise			2 h after exercise			T	Delta
	Mean	±	SD	Mean	±	SD	p-value	%
Leukocytes (10^3/µL)								
EIB−	4.077	±	1.065	4.852	±	1.088	0.074	19.00
EIB+ Salb	4.103	±	1.047	4.425	±	1.120	0.345	7.4
Lymphocytes (10^3/µL)								
EIB−	1.837	±	0.565	1.690	±	0.610	0.346	-8.00
EIB+ Salb	1.787	±	0.398	1.428	±	0.471	0.075	-20.08
Monocytes (10^3/µL)								
EIB−	0.488	±	0.369	0.288	±	0.168*	0.046	-40.98
Granulocytes (10^3/µL)								
EIB−	1.747	±	0.601	2.870	±	0.962*	0.027	64.28
EIB+ Salb	1.903	±	1.148	2.675	±	1.085*	0.028	40.56
Neutrophils (10^3/µL)								
EIB−	1.729	±	0.595	2.841	±	0.953*	0.028	64.31
EIB+ Salb	1.884	±	1.137	2.648	±	1.074*	0.027	40.55
Eosinophils (10^3/µL)								
EIB−	0.012	±	0.004	0.020	±	0.007*	0.028	66.66
Basophils (10^3/µL)				.				
EIB−	0.005	±	0.002	0.009	±	0.003*	0.027	80.00
EIB+ Salb	0.006	±	0.003	0.008	±	0.003*	0.028	33.33

EIB−: group not susceptible to exercise-induced bronchospasm; EIB+ Salb: group susceptible to exercise-induced bronchospasm treated with salbutamol; T: wilcoxon test; Delta %: variation in percentage of the values recorded 2 hours after exercise compared to the values obtained before exercise in the subjects of the EIB− group and those of the EIB+ Salb group; *: $p < 0.05$; SD: Standard Deviation.

To examine the variation in blood leukocyte subsets concentrations, we compared the mean concentrations obtained before and 2 hours after exercise in the EIB− and EIB+ Salb groups (Table 5(b)). In both groups, the numbers of granulocytes, neutrophils and basophils obtained 2 hours after exercise were significantly higher than those recorded before exercise. The changes in the leukocyte and lymphocyte concentrations before and 2 hours after exercise were not significantly different between the two groups (Table 5(b)).

4. Discussion

The mean post-exercise FEV1 value recorded in athletes who were sensitive to EIB and previously treated with salbutamol (EIB+ Salb) was not significantly different from that obtained in nonsensitive athletes (EIB−) (Table 1). Nsompi *et al.* [14] showed that EIB+ athletes without prior Salbutamol treatment had a

significantly lower mean post exercise FEV1 value (−18.37%) than their EIB− counterparts. The results of the current study highlighted the effects of salbutamol on EIB symptoms in the EIB+ Salb athletes. Anderson [6] showed that the inhalation of a β2-agonist at the recommended dose immediately before exercise effectively alleviated the symptoms of EIB by stimulating β2 receptors on mast cells, which inhibits the release of contractile mediators and induces airway smooth muscle relaxation, thereby acting as an antagonist of these mediators. Salbutamol is a short-acting β2-agonist that can prevent and treat the symptoms of EIB in athletes with or without a diagnosis of asthma [8]. According to Prenner [15], salbutamol is a commonly used drug in the treatment of bronchial asthma. Moreover, short-acting β2-agonists inhaled before training can attenuate EIB in 80% - 95% of patients with EIB, and their efficacy lasts for 2-3 hours of exercise [7].

The anthropometric characteristics were not significantly different between the EIB+ Salb athletes and EIB− athletes (**Table 1**). These results show that, initially, the subjects of the two groups were physiologically similar. Before exercise, the mean concentrations of inflammatory cells and IL-6 did not significantly differ between the two groups (**Table 2**), indicating that the immune function in both groups was statistically similar at baseline. The average concentrations of systemic inflammatory cells recorded before exercise, immediately after exercise and 2 hours after exercise revealed significant differences in leukocytes, lymphocytes, total granulocytes, neutrophils and basophils in the EIB+ Salb group. Similar results were observed for the EIB− group (**Table 3**). These results showed that both groups of athletes had similar mobilizations of inflammatory cells in response to physical exertion. This can be explained by the fact that salbutamol attenuated EIB. Several studies have suggested that β2-adrenergic receptors play a role in suppressing inflammation, as stimulation of these receptors produces anti-inflammatory effects [16] [17]. The anti-inflammatory effects of β2-adrenergic receptors in models of pulmonary inflammation [18] have confirmed the role of β2-adrenergic receptors in inflammatory states.

In the EIB+ Salb group, the concentrations of monocytes and eosinophils before exercise, immediately after exercise and 2 hours after exercise were not significantly different, whereas there were significant variations in these concentrations in the EIB− group (**Table 3**). This result could be explained by the anti-inflammatory effects of salbutamol. Romberger *et al.* [10] found that salbutamol induced a reduction in pro inflammatory cytokines and inhibited the recruitment of inflammatory cells in mice exposed to organic dust. Tiina Keränen *et al.* [19] showed that salbutamol increased the expression of mitogen-activated protein kinase phosphatase-1 (MKP-1), which is an endogenous suppressor of the inflammatory response. However, EIB is an eosinophilic pulmonary inflammation, and Yoshikawa *et al.* [20] reported that the severity of EIB is associated with eosinophilic airway inflammation. Additionally, it has been reported that blood eosinophil concentrations may be useful in predicting the presence or severity of EIB [21]. Salbutamol acts on β2-adrenergic receptors located on the

surface of inflammatory cells, including monocytes, macrophages and eosinophils, to attenuate the inflammation induced by EIB. In the current study, salbutamol pretreatment in the EIB+ group may have played a role in inhibiting the expression of systemic monocytes and eosinophils.

The mean concentrations of leukocytes and systemic lymphocytes recorded immediately after exercise were significantly higher (57.44% and 108.28%, respectively) than those obtained before exercise in the EIB+ Salb group (**Table 5(a)**). In the EIB− group, compared with the values obtained before exercise, a significant increase in the mean values of leukocytes (46.3%) and lymphocytes (81.98%) immediately after exercise was also observed (**Table 5(a)**). These results revealed leukocytosis and post-exercise lymphocytosis in both groups. The observed leukocytosis can be explained by the increase in circulating lymphocytes [22] [23]. Lymphocytosis is proportional to the intensity and duration of exercise, with an increase in the number of T lymphocytes and, to a lesser extent, B lymphocytes, compared with the numbers before exercise [24] [25]. The mobilization of these cell subgroups (T and B) is largely influenced by the action of catecholamines, which are released by the nervous system in response to increased energy demand [26].

In the EIB+ Salb group, the mean values of total granulocytes, neutrophils and basophils before and immediately after exercise were not significantly different (**Table 5(a)**). Similar results were observed in the EIB− group (**Table 5(a)**). In the EIB+ Salb group, this result could be explained by pretreatment with salbutamol. Tahereh Eteraf-Oskouei *et al.* [11] showed that intravenous administration of salbutamol inhibited the accumulation of inflammatory cells during inflammation. Moreover, the results of previous studies have shown that immune cells such as monocytes and lymphocytes express adrenergic receptors that because activated can exhibit anti-inflammatory effects [27] [28]. The two groups in the current study presented similar results, and the intensity, duration and type of effort could explain these results. Physical exercise has inflammatory [29] and anti-inflammatory [30] effects.

In addition, in the EIB+ Salb group, the mean leukocyte and lymphocyte concentrations observed before and 2 hours after exercise were not significantly different (**Table 5(b)**). Similar results were seen in the EIB− group (**Table 5(b)**). These results indicated a return to the initial leukocyte and lymphocyte concentrations 2 hours after exercise. Our results corroborate those of Walsh *et al.* [24], who showed that leukocyte concentrations returned to their initial level a few hours after the end of exercise.

Compared with the numbers before exercise, the numbers of total granulocytes (40.56%), neutrophils (40.55%) and basophils (33.33%) recorded 2 hours after exercise were significantly greater in the EIB+ Salb group (**Table 5(b)**). The same trends were observed in the EIB− group (**Table 5(b)**). These results corroborate those of Fortunato *et al.* [31] who observed a significant increase in neutrophils up to 2 hours after exercise in trained and untrained subjects. Cannon *et al.* [32] demonstrated an increase in the number of circulating neutrophils after

eccentric exercise (45 minutes at 78% of maximum heart rate) in 12 subjects aged 61 - 72 years and in 9 subjects aged 20 - 32 years.

Kalsen *et al.* [33] treated swimmers with or without airway hyperreactivity with a combined inhalation of β2-agonists (salbutamol, formoterol and salmeterol) and observed a post-exercise increase in IL-6 in both groups. However, we found that after inhalation of salbutamol, the plasma concentrations of IL-6 before exercise, immediately after exercise and 2 hours after exercise were not significantly different in the EIB+ Salb group (**Table 3**). Or in the EIB− group (**Table 3**). These results could be explained by the duration, intensity and type of exercise. Fisher [34] reported that post-exercise variations in IL-6 depend on the duration, intensity and type of exercise.

5. Conclusion

This study showed that in response to physical exercise, the systemic immune responses in the EIB+ Salb athletes were similar to those observed in the EIB− athletes. By attenuating inflammation, salbutamol inhibited the occurrence of EIB by reducing the proliferation of inflammatory cells. In this way, salbutamol restored the immune status of the EIB-susceptible athletes.

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Authors' Contribution

Nsompi Florent wrote the research protocol, participated in the data collection, carried out the data processing and wrote the manuscript.

Messan Folly and Boussana Alain Marc brought relevant criticisms at the level of the research protocol, and corrected the manuscript.

Mabounda Kounga Paul Roger and Moussouami Simplice Innocent contributed to the drafting of the research protocol and participated in the data collection.

Bouhika Eddie Janvier participated in the data entry and editing of the manuscript.

Tito Albérick proofread the manuscript in order to bring relevant criticism concerning the bibliographical review.

Ethical Considerations

This study was approved by the Scientific Council of the Higher Institute of Physical and Sports Education of Marien Ngouabi University in the Republic of Congo in accordance with the 1975 Helsinki Declaration relating to ethics.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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