ISSN: 0975-833X

Availableonlineathttp://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 05, pp.3624-3631, May, 2019

DOI: https://doi.org/10.24941/ijcr.35337.05.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

COMPARISON OF IMMUNE CELLS AND IL-6 VARIATIONS OBSERVED BEFORE AND AFTER EXERCISE-INDUCED BRONCHOSPASM IN ELITE ENDURANCE ATHLETES

[1]Florent Nsompi, [*,1]Folly Messan, [2]Tchazou Kpatcha, [3]Fabien R. Niama and [4]Mohamed M. Lawani

[1]Laboratory of Respiratory, Hormonal and Gerontological Explorations of the Sportsman, National Institute of Youth, Physical Education and Sport (INJEPS), University of Abomey-Calavi, (Benin)
[2]Laboratoire de Physiologie, Pharmacologie, Faculté Des Sciences, Université de Lomé, (Togo)
[3]Laboratoire de biologie moléculaire, Faculté des Sciences et Techniques, Université Marien Ngouabi. Brazzaville, (Congo)
[4]Laboratory of Biomechanics and Performance (LaBioP), University of Abomey-Calavi (BENIN) - National Institute of Youth, Physical Education and Sport (INJEPS)

ARTICLEINFO

Article History:
Received 08th February, 2019
Received in revised form
24th March, 2019
Accepted 10th April, 2019
Published online 30th May, 2019

Key Words:
Bronchospasm, immune system.

Corresponding author: Folly Messan

ABSTRACT

Background: Exercise-induced bronchospasm (EIB) is a constriction of the bronchi that occurs in response to dehydration of the airways. EIB is characterized by a very common inflammatory condition in athletes. Therefore, the present study aims to assess the variations in circulating inflammatory cells and interleukin-6 (IL-6) levels before and after EIB in a population of endurance athletes. **Materials and Methods:** The study involved 16 athletes (23.56 ± 3.55 years, 171.62 ± 6.81 cm and 60.68 ± 5.53 kg). To determine the prevalence of EIB, the subjects were subjected to a laboratory stress test that consisted of a continuous treadmill run from 7.5 Km.h-1 with an increment of 1.5 Km .h-1 every 3 minutes until exhaustion. Respiratory functional explorations were carried out before and at 5 minutes after the effort using a Spirobank G spirometer. Blood samples were taken before exercise, immediately after exercise and at 2 hours after exercise, and EIB diagnosis was based on a reduction in blood pressure of at least 10% of the first-second forced expiratory volume (FEV1) in relation to the pre-exercise value. Subjects who presented this criterion were determined as sensitive to bronchospasm and formed the EIB (+) group. Subjects who were not identified as such were considered non susceptible and formed the EIB (-) group. Inflammatory cell counts were performed using an Elite3 automated system, and IL-6 plasma concentrations were determined using an IL-6 ELISA kit. Results: The prevalence of EIB was 37.5%. A significant mean decrease in the post exercise FEV1 of 16.31% was observed in the EIB (+) group compared with that in the EIB (-) group, confirming EIB in these individuals. For leukocytes and lymphocytes, the mean values were recorded immediately after exertion, and those obtained before exertion showed no significant difference ($p > 0.05$) in EIB (+) athletes, whereas in EIB (-) athletes, the mean values of leukocytes and lymphocytes observed immediately after exertion were significantly higher ($p < 0.05$) than those recorded before exercise. In the EIB (+) group, the mean concentrations of total granulocytes, neutrophils, eosinophils, basophils and IL-6 observed immediately after exertion were increased significantly ($p < 0.05$) compared to those measured before the effort . The mean leukocyte values recorded at 2 hours after exercise were significantly higher than those obtained before exercise in EIB (+) athletes. In the EIB (+) group, the mean value of the lymphocytes observed at 2 hours after exercise and the value recorded before exercise did not show a significant difference ($p > 0.05$), while in the EIB (-) group, the average value of lymphocytes obtained at 2 hours after exertion was significantly lower than the value recorded before exercise. The mean concentrations of total granulocytes, neutrophils, eosinophils, and basophils observed at 2 hours after exercise were significantly increased ($p < 0.05$) compared with those obtained before exercise in EIB (+) athletes. **Conclusion:** The occurrence of exercise-induced bronchospasm mobilizes a significant increase in inflammatory cells and IL-6 levels. Therefore, the immune system of athletes sensitive to EIB is strongly solicited.

Citation: Florent Nsompi, Folly Messan, Tchazou Kpatcha, Fabien R. Niama and Mohamed M. Lawani. **2019.** "Comparison of immune cells and il-6 variations observed before and after exercise-induced bronchospasm in elite endurance athletes", *International Journal of Current Research*, 11, (05), 3624-3631.

INTRODUCTION

Exercise-induced bronchospasm (EIB) is a sudden constriction of the airway that occurs in sensitive subjects at the end of exercise in response to dehydration of the airways. The severity or severity of this response reflects the sensitivity of bronchial smooth muscle cells to inspired air quality, inspired air pollutant concentrations, and exercise intensity (Rundell *et al*., 2013; Rundell *et al*., 2015; Rundell *et al*., 2018). EIB prevalence values are higher for athletes (11-59%) than for sedentary individuals (4-20%) (Bussotti*et al*., 2014; Burnett *et al*., 2016; Becerril-Ángeles *et al*., 2017; Messan *et al*., 2017; Caggiano *et al*., 2017). These increasingly high EIB prevalence values are observed in endurance sports under cold

air conditions (Burnett etal, 2016; Messan *et al*., 2017; Bonini, 2018; Mahmoud *et al*., 2013; Robson-Ansley *et al*., 2012; Larsson *et al*., 1993) and under hot and humid air conditions (Mahmoud *et al*., 2013; NSOMPI *et al*., 2018; Ouattara *et al*., 2012; Messan *et al*., 2011). Ambient air pollutants are present in sports venues, where athletes are chronically exposed (Rundell *et al*., 2018; (Mohammadreza Modaresi *et al*., 2015). During training or competitions, endurance athletes face high levels of intensity. Indeed, these athletes are subject to a training volume of at least 5 hours per week and a maximum oxygen consumption of up to 65 mL. min^{-1}. kg^{-1} (Schild *et al*., 2016). This large amount of air frequently ventilated by endurance athletes under unfavorable environmental conditions is likely to cause respiratory tract damage. In addition, the micro lesions generated by hyperventilation are responsible for cell permeability to Na^{+}, Cl^{-}, K^{+} and Ca^{2+} ions. These aberrations induce the release of chemical mediators involved in the initial inflammatory process of EIB (Hallstrand *et al*., 2013; Kippelen *et al*., 2010). Studies have indicated that histamine, cysteinyl leukotrienes and prostaglandins are chemical mediators responsible for the occurrence and development of EIB in the airways (Hallstrand *et al*., 2013; Hallstrand *et al*., 2005).

These chemical mediators are released by inflammatory cells, including mast cells, eosinophils, and neutrophils (Hallstrand *et al*., 2005; Joshi *et al*., 2013). EIB is the consequence of bronchoconstriction and transient bronchial inflammation in response to exercise, but conversely, exercise can be viewed as the exacerbation of hyper reactivity and bronchial inflammation in sensitive people. Bonini and Silvers (2018) reported that exercise is a causative factor of airway inflammation. EIB is a functional disruption of the body that involves the immune system. Our hypothesis was that in a seizure situation, the immune system is much more involved in EIB-sensitive athletes than in non susceptible athletes. Therefore, the present study aims to assess the circulating inflammatory cell and circulating IL-6 variations observed before and after EIB in the runner population.

MATERIALS AND METHODS

Framework of the study: This study is a prospective study conducted in June 2018 among Congolese athletes residing in Brazzaville, the political capital of the Congo located in Central Africa. The study was conducted in the weight room of the national football training center "STADE MASSAMBA DEBAT". The laboratory analyses were carried out at the "Louis Pasteur" National Laboratory in Brazzaville.

Topics of the study: The study population consisted of cross-country runners, all male. The anthropometric characteristics of the subjects are presented in Table 1. These athletes regularly take part in national and international competitions in the 5000-meter race, 10000-meter race and international half marathon organized each year by the Congolese Athletics Federation. The target population is composed of athletes whose performances are among the top 10 in each event category. The subjects of the study must be holders of a license from the Congolese Federation of Athletics and reside in the city for at least 5 years. Athletes who did not agree by providing signed informed consent and those whose spirometry results did not meet the criteria were excluded from the study. Similarly, athletes recognized as smokers or those who had symptoms of asthma and respiratory conditions duly noted by clinical examinations were excluded.

Sampling: The top 10 performances in each long distance race category (5000 meters, 10000 meters and half marathon) constituted a preselection of 50 athletes, 16 of whom were selected on the basis of the regularity of their best performances.

Equipment: A Stanley tape measure, graduated from 0 to 200 cm, was placed against a support in the vertical plane to measure the size of the subjects standing barefoot; a SECA brand bathroom scale with a flat dial scale calibrated in kilograms and placed in a perfectly horizontal plane was used to determine the body mass of the subjects. At the beginning of the evaluation, the scale was calibrated using a mass of one kilogram to ensure the reliability of the measurements. The measurement range of the scale ranged from 0 to 150 kilograms, and the measurement accuracy was plus or minus 10 grams. Respiratory functional exploration was performed using a CE Spirobank G portable spirometer, a product of "Medical International Research (MIR)" (volume accuracy: ± 3% or 50 ml, flow accuracy: ± 5% or 200 ml / s) and using single-use nozzles. The Spirobank G spirometer is composed of a central unit, a monitor, a turbine and WinspiroPRO software installed on the central unit. The "RS232" cable transmits in real time to the central unit and instantly displays on the monitor, the results of the subject's respiratory explorations in terms of volume flow curve. Next, a Matrix treadmill (model: T-3X-04-C S / N: CTM 523140306138) was used to submit the subjects to stress tests.

Experimental protocol: In the first phase of the experiment, the selected athletes completed the individual information sheets and signed the informed consent forms. Body mass and height were measured. In the second phase, measurements of volumes and pulmonary flow were performed after the subject, while standing, blew into the turbine, and the blood samples were obtained in a sitting position. The subject then carried out the exercise test on a treadmill with a brisk walk at the beginning and then trotting along at a speed of the mat set at 7.5 $Km.h^{-1}$, followed by an incremented stroke of 1.5 $Km.h^{-1}$ every 3 minutes until exhaustion. At the end of the test, breath tests were performed after 5 minutes, and the blood samples were collected immediately after and at 2 hours after the test.

Modalities of functional exploration tests: Functional respiratory tests (FRT) were performed under the supervision of a medical technician. After the acquisition of height, body mass, age, sex and race, the spirometer central unit automatically calculates the theoretical values of each respiratory parameter. During the respiratory functional explorations (RFE), the subject, while standing, with pinched nose and holding the turbine between two hands, breathes naturally and calmly through the mouthpiece connected to the device of the spirometer. The athlete is then instructed to fill his lungs as much as possible by inhaling air and then emptying the air into the turbine as quickly as possible in a continuous and complete manner for a maximum of 6 seconds. At the end of the test, the best test was selected from three reproducible results that have been validated according to the spirometer algorithms. Peak volumes and flow rates were recorded.

Prerequisites for the stress test: Participants in this study were advised to abstain from all sports for 48 hours prior to

testing and to avoid consuming alcohol, coffee or medication on the day of testing.

Stress test: The exercise test consisted of continuous treadmill exercise at a speed that was increased every 3 minutes by 1.5 $Km.h^{-1}$ from 7.5 $Km.h^{-1}$ until exhaustion. Exhaustion was noted by the inability of the subject to maintain a speed consistent with that of the mat. The race was conducted without prior warm-up. All subjects performed stress tests without encouragement and under the supervision of three PSE teachers and a doctor with his two assistants. A defibrillator was available in cases of cardiac malaise. Due to possible influences on the performance of the subject, all stress tests were performed in the mornings between 7:30 and 9:30, and food intake was not allowed before and during this time period. The resuscitation unit of the hospital was informed of the experimentation. The present study was authorized by the Scientific Council of the Higher Institute of Physical and Sports Education (HIPSE) of the Marien Ngouabi University of Congo-Brazzaville in accordance with the 1975 Helsinki Declaration on Ethics.

Sampling and Analysis of Whole Blood and Plasma Samples: Venous blood from the forearm of each subject was collected into a tube containing EDTA as anticoagulant and then transferred to the laboratory within 2 hours for analysis. Examination of the blood count was performed by automaton (Elite 3, China). To isolate the plasma, the blood in the EDTA tubes was centrifuged at 5000 rpm (revolution per minute) for 5 minutes using a centrifuge (UNIVERSAL 320, Hettich). The collected plasma was transferred to 2 ml cryotubes previously identified under the hood. The cryotubes were then placed in the boxes and stored at -20 °C. The collected plasma supernatant was used for the quantification of IL-6 using a human IL-6 ELISA kit (Aviva Systems Biology, San Diego, USA) according to the manufacturer's instructions.

Variables studied: The diagnosis of bronchospasm in a subject was positive if the post exercise FEV1 decreases by at least 10% from the resting value by applying the formula Delta FEV1 (%) = [(FEV1 postexercise x 100) / (VEMS rest)] - 100. At the end of the exercise test, the mean post exercise FEV1 of the EIB (+) group was compared with that of the EIB (-) group. The status of each subject in relation to his sensitivity to EIB [EIB (-) or EIB (+)] and the timing of the measurements (pre-effort and post exercise) constituted independent variables. Inflammatory cells and IL-6 levels were dependent variables.

Statistical analysis: Descriptive statistics were used to generate the means and standard deviations of the sensitive group [EIB (+)] and no susceptible group [EIB (-)] samples. The Mann-Whitney test was used to compare mean values. Anthropometric, respiratory, physical fitness level and mean values of inflammatory cells and pre-exercise IL-6 levels between the EIB (+) group and the EIB (-) group. The nonparametric Wilcoxon test compared the mean values of the inflammatory cell and IL-6 variables recorded before exercise and after exercise. The variables were recorded and processed using Stat View 5 software (version 5) from Abacus Concepts Inc. (Berkeley, CA, USA). The significance was set at $p < 0.05$.

RESULTS

The anthropometric, respiratory and performance results of the EIB (-) and EIB (+) group runners are presented in Table 1. Of the 16 athletes, 6 were found to be sensitive to bronchospasm, with a 37.5% prevalence of EIB. A significant mean fall in post exercise FEV1 of 16.31% was observed in EIB (+) compared with EIB (-) athletes (Table 1). The mean values of inflammatory cells and IL-6 levels recorded before exercise in the BIE (-) group did not differ significantly from those obtained before exercise in the EIB (+) group (Table 2). This result reflects the equivalence of the two groups with respect to the dependent variables. For leukocytes and lymphocytes, the mean values recorded immediately after exertion and those obtained before exertion showed no significant difference ($p > 0.05$) in EIB (+) athletes, whereas in EIB (-) athletes, the mean values of these same immune parameters observed immediately after exertion were significantly higher (21.38% and 36.94%, respectively) than those recorded before exercise (Table 3).

The average value of monocytes obtained immediately after exercise and that recorded before exercise did not show a significant difference ($p > 0.05$) in the athletes of the two groups. In the EIB (+) group, the mean concentrations of total granulocytes, neutrophils, eosinophils, basophils, and IL-6 observed immediately after exertion were significantly higher (13.14%, 13.14%, 9. 09%, 12.76% and 24.77%, respectively) than those obtained before exertion, while in the EIB (-) group, the average concentrations of these same immune parameters recorded immediately after exertion and those obtained before exertion were not significantly different ($p > 0.05$) (Table 3). The average value of leukocytes recorded at 2 hours after exertion was significantly higher (22.96%) than that obtained before exertion in EIB (+) athletes, whereas in EIB (-) athletes, the average value of leukocytes obtained at 2 hours after effort and that recorded before exercise did not show a significant difference ($p > 0.05$) (Table 4). In the EIB (+) group, the mean value of the lymphocytes observed at 2 hours after exercise and that recorded before exercise did not show any significant difference ($p > 0.05$), whereas in the EIB (-) group, the mean value of the lymphocytes obtained at 2 hours after exertion was significantly lower (29.23%) than that recorded before exercise (Table 4). The mean concentrations of total granulocytes, neutrophils, eosinophils, and basophils observed at 2 hours after exercise were significantly higher (55.87%, 55.86%, 54.54%, and 48.93%, respectively) than those obtained before effort in EIB (+) athletes, whereas in EIB (-) athletes, the average concentrations of these same immune parameters recorded at 2 hours after effort and those obtained before exercise did not show a significant difference ($p > 0.05$) (Table 4).

DISCUSSION

The aim of this work was to assess the systemic variations of inflammatory cells and IL-6 mobilized after bronchospasm is induced by a bronchial stress provocation test performed in runners. The prevalence of runners diagnosed with EIB [EIB (+) group] was 37.5%, a value similar to that observed in a survey of Ivorian athletes that estimated the prevalence of EIB (+) at 42.6% (Ouattara*et al*., 2012). In this study, the competition intensity levels were high, and the estimated maximum oxygen uptake (VO2 max) was 58.51 ml. kg^{-1}. min^{-1}. This value indicates a large volume of air during training and competition, and the airways are progressively exposed to bronchial hyperosmolarity. During exercise periods, water is lost to the surface of the respiratory tract by evaporation, which dehydrates airway surfaces and triggers the events

Table 1. Comparison of anthropometric, respiratory and performance characteristics between BIE (-) and BIE (+) subjects

	Total Group (n = 16)	EIB (-) (n = 10)	EIB (+) (n = 6)	(C) Statistical test	(D) Delta
	Mean ± SD	Mean ± SD	Mean ± SD	p value	%
Age (an)	23,56 ± 3,56	23,50 ± 3,92	23,67 ± 3,20	p > 0.05	0,71
Height (cm)	171,63 ± 6,81	173,00 ± 6,46	169,33 ± 7,34	p > 0.05	-2,12
Weigiht (kg)	60,69 ± 5,53	62,20 ± 4,39	58,17 ± 6,71	p > 0.05	-6,48
BMI (kg/m^2)	20,62 ± 1,71	20,81 ± 1,39	20,31 ± 2,25	p > 0.05	-2,37
FEV1_(Before) (L)	3,65 ± 0,53	3,70 ± 0,54	3,57 ± 0,55	p > 0.05	-3,43
FEV1_(After) (L)	3,52 ± 0,57	3,75 ± 0,51	3,14 ± 0,46*	p > 0.05	-16,31
Duration (min)	26,01 ± 2,95	26,33 ± 3,37	25,48 ± 2,26	p > 0.05	-3,25
VO_2 max (ml / min / kg)	58,51 ± 3,37	58,21 ± 4,04	59,02 ± 2,04	p > 0.05	1,40

BMI: Body Mass Index; FEV1_ (Before): Forced expiratory volume per second before exercise (mean values); FEV1_ (After): Forced expiratory volume per second after exercise; EIB (-): subjects whose FEV1 has not decreased by at least 10% of the baseline; EIB (+): subjects whose FEV1 decreased by at least 10% compared to baseline; (C) Test: comparison of mean values between BIE (-) and EIB (+); (D) Delta%: Percentage change in EIB (+) group values compared to EIB (-) group values; VO2 max: Maximum oxygen consumption. * : p< 0.05

Table 2. Comparison of the circulating inflammatory cells and IL-6 average concentrations recorded before exercise between EIB (-) and EIB (+) subjects

	EIB (-) (n = 10)	EIB (+) (n = 6)	Statistical test
	Mean ± SD	Mean ± SD	p value
Leukocytes (10^3 / µL)	4,961 ± 1,146	4,593 ± 2,2796	0,33
Lymphocytes (10^3 / µL)	2,244 ± 0,797	2,403 ± 1,374	0,70
Monocytes (10^3 / µL)	0,597 ± 0,410	0,615 ± 0,472	0,66
Granulocytes (10^3 / µL)	2,120 ± 0,899	1,575 ± 0,561	0,29
Neutrophils (10^3 / µL)	2,099 ± 0,890	1,559 ± 0,556	0,29
Eosinophils (10^3 / µL)	0,015 ± 0,006	0,011 ± 0,004	0,29
Basophils (10^3 / µL)	0,006 ± 0,003	0,005 ± 0,002	0,29
IL-6 (pg / ml)	0,115 ± 0,036	0,109 ± 0,018	1,00

EIB (-): Group of "no sensitive" subjects to exercise-induced bronchospasm; EIB (+): Group of "sensitive" subjects with exercise-induced bronchospasm; T: Mann Whitney test. P values > 0.05.

Table 3. Comparison of leukocyte and IL-6 blood concentrations recorded before and after exercise in EIB (-) group (n = 10) and EIB (+) group (n = 6)

	Before Stress test	After Stress test	Statistical test	Delta
	Mean ± SD	Mean ± SD	p value	%
Leukocytes (10^3 / µL)				
EIB(-)	4,961 ± 1,146	6,022 ± 1,426	0,019*	21,38
EIB(+)	4,593 ± 2,2796	5,662 ± 1,967	0,075	23,27
Lymphocytes (10^3 / µL)				
EIB(-)	2,244 ± 0,797	3,073 ± 1,350	0,0218*	36,94
EIB(+)	2,403 ± 1,374	3,265 ± 1,203	0,116	35,87
Monocytes (10^3 / µL)				
EIB(-)	0,597 ± 0,410	0,724 ± 0,4828	0,213	21,27
EIB(+)	0,615 ± 0,472	0,618 ± 0,4228	0,500	0,48
Granulocytes (10^3 / µL)				
EIB(-)	2,120 ± 0,899	2,223 ± 1,019	0,126	4,85
EIB(+)	1,575 ± 0,561	1,782 ± 0,642	0,0277*	13,14
Neutrophils (10^3 / µL)				
EIB(-)	2,099 ± 0,890	2,201 ± 1,009	0,126	4,85
EIB(+)	1,559 ± 0,556	1,764 ± 0,636	0,0277*	13,14
Eosinophils (10^3 / µL)				
EIB(-)	0,015 ± 0,006	0,016 ± 0,007	0,126	6,66
EIB(+)	0,011 ± 0,004	0,012 ± 0,004	0,0277*	9,09
Basophils (10^3 / µL)				
EIB(-)	0,006 ± 0,003	0,007 ± 0,003	0,126	16,66
EIB(+)	0,005 ± 0,002	0,005 ± 0,002	0,0277*	12,76
IL-6 (pg / ml)				
EIB(-)	0,115 ± 0,036	0,125 ± 0,034	0,575	8,69
EIB(+)	0,109 ± 0,018	0,136 ± 0,026	0,046*	24,77

EIB (-): Subjects of the non sensitive group to exercise-induced bronchospasm; EIB (+): Subjects of the sensitive group to exercise-induced bronchospasm; IL-6: Interleukin – 6; Delta%: Percentage change in values obtained immediately after exercise compared to values recorded before exercise in EIB (-) and EIB (+). * : p < 0.05.

leading to the contraction of bronchial smooth muscle (Weiler *et al*., 2016). A mast cell-mediated release of prostaglandins (prostaglandin D2), leukotrienes, histamine and tryptase occurs. These signaling molecules mediate the contraction of airway smooth muscle and increase mucus production and sensory nerve activation, and their release is considered to be the main stimulant of bronchospasm (Caggiano *et al*., 2013; Weiler *et al*., 2016; Cavaleiro Rufo *et al*., 2018; Kurowski *et al*., 2018).

Exercise-induced hyperventilation causes bronchial epithelial microlysis, resulting in an influx of eosinophils and neutrophils into the Broncho alveolar lavage fluid (Del Giacco *et al*., 2015). In addition, the micro lesions generated by hyperventilation are responsible for cell permeability to Na^+, Cl^-, K^+ and Ca^{2+} ions (Messan *et al*., 2017; Colbey *et al*., 2018). These aberrations induce the release of chemical mediators involved in the initial inflammatory process of EIB and EIA.

Table 4. Comparison of total blood leukocyte and plasma IL-6 concentrations recorded before and 2 hours after exercise in EIB (-) (n = 10) and EIB (+) groups (n = 6)

	Before Stress test			2 hours After Stress test			Statistical test	Delta
	Mean	±	SD	Mean	±	SD	p value	%
Leukocytes (10^3 / µL)								
EIB(-)	4,961	±	1,146	4,837	±	1,173	0,959	-2,49
EIB(+)	4,593	±	2,279	5,648	±	1,998	0,046*	22,96
Lymphocytes (10^3 / µL)								
EIB(-)	2,244	±	0,797	1,588	±	0,635	0,0129*	-29,23
EIB(+)	2,403	±	1,374	2,502	±	1,336	0,846	4,11
Monocytes (10^3 / µL)								
EIB(-)	0,597	±	0,41	0,614	±	0,62	0,767	2,84
EIB(+)	0,615	±	0,472	0,693	±	0,413	0,240	12,68
Granulocytes (10^3 / µL)								
EIB(-)	2,120	±	0,899	2,635	±	1,307	0,203	11,45
EIB(+)	1,575	±	0,561	2,455	±	1,181	0,0277*	55,87
Neutrophils (10^3 / µL)								
EIB(-)	2,099	±	0,890	2,609	±	1,294	0,203	24,29
EIB(+)	1,559	±	0,556	2,430	±	1,170	0,0277*	55,86
Eosinophils (10^3 / µL)								
EIB(-)	0,015	±	0,006	0,018	±	0,009	0,203	20,00
EIB(+)	0,011	±	0,004	0,017	±	0,008	0,0277*	54,54
Basophils (10^3 / µL)								
EIB(-)	0,006	±	0,003	0,008	±	0,004	0,203	33,33
EIB(+)	0,005	±	0,002	0,007	±	0,004	0,0277*	48,93
IL-6 (pg / ml)								
EIB(-)	0,115	±	0,036	0,106	±	0,025	0,475	-7,82
EIB(+)	0,109	±	0,018	0,125	±	0,140	0,141	14,67

EIB (-): Group of no sensitive subjects to exercise-induced bronchospasm; EIB (+): Group of sensitive subjects to exercise-induced bronchospasm; T: Wilcoxon test; Delta% : percentage change in the values observed 2 hours after effort compared to the values obtained before effort in EIB (-) and EIB (+). * $p < 0.05$

Physical exercise is a stress that causes the triggering process of airway inflammation and systemic inflammation. Inflammation induces an increase in inflammatory cells and pro- and anti-inflammatory cytokines by activating the innate and adaptive immune system.

After the Stress Test: In this study, intragroup comparisons of the results were performed. In EIB (+) subjects, a significant increase of 13.14% granulocytes, 12.95% neutrophils, 9.09% eosinophils and 12.76% basophils was observed immediately after exercise compared to the pre-exercise values, whereas in EIB (-) subjects, a significant increase of 21.38% leukocytes and 36.94% lymphocytes was observed immediately after exercise compared to the pre-effort values (Table 3). These results revealed granulocytosis, neutrophilia, eosinophilia and basophilia at the end of the effort in EIB (+) subjects and leukocytosis and end-of-effort lymphocytosis in EIB (-) subjects. Through these results, we found that in response to physical effort, the variations in the inflammatory cells tested were different in the subjects of the two groups. The result in EIB (+) subjects could be explained by the presence of EIB initiated by inflammation. According to Hallstrand *et al.* (2013), Del Giacco *et al.* (2015) and Caggiano *et al.* (2017), the activation of inflammatory cells, including neutrophils, eosinophils, and mast cells, is observed during EIB. Activation of these inflammatory cells results in the release of inflammatory mediators, such as cysteinyl leukotrienes, prostaglandin, and histamine. In addition, EIB occurs during or shortly after exercise and is usually at peak levels at 5 to 10 minutes after full exercise and usually ceases at approximately 60 minutes thereafter (Aggarwal *et al*., 2018; Holzer *et al*., 2006). In EIB (-) subjects, however, the significant increase in mean leukocyte concentrations can be explained by the number of total lymphocytes in circulation and the significant increase in mean concentrations of lymphocytes was mediated by catecholamines (Hansen *et al*., 1991; Bishop, 2006). In addition, Ostrowski *et al.* (1999) showed that intense and prolonged exercise induces systemic inflammation.

In addition, intense training dramatically increases IL-6 up to 100-fold, and the peak level of IL-6 is reached at the end of exercise or shortly thereafter, at approximately 30 minutes after exercise (Fischer, 2006; Leggate *et al*., 2010; Pedersen and Febbraio, 2012). This fact has already been demonstrated in response to endurance exercise (Schild *et al*., 2016; Scherr *et al*., 2011; Nieman *et al*., 2007; Perry *et al*., 2013) and high intensity interval exercise (Meckel *et al*., 2009; Zweetsloot *et al*., 2014). However, our work has shown a significant increase of 24.77% in the average level of IL-6 observed immediately after exercise compared to the average pre-exercise rate in EIB (+) athletes, whereas in their EIB (-) counterparts, the IL-6 variations observed between the mean value immediately after exertion and that before exertion did not show a significant difference (Table 3). These results also show that significant changes in plasma levels of IL-6 immediately after physical exertion occurred only in EIB-sensitive athletes.

Thus, in EIB (+) athletes, would EIB-induced inflammation in response to physical exertion be responsible for the post exercise increase in plasma IL-6? Would exercise-induced systemic inflammation be the source of the increase in plasma IL-6 at the end of the effort? Would exercise-driven muscles be the source of the post exercise increase in IL-6 plasma levels? Notably, physical exercise, by type, intensity, and duration (Cruzat *et al*., 2014; Bruunsgaard *et al*., 1997), causes micro muscle damage, triggering local and systemic inflammation with the release of pro- and anti-inflammatory cytokines (Karalaki *et al*., 2013). In addition, there is an interaction between muscle lesions and increased levels of IL-6, and Toigo *et al* (2006) and Croisier *et al* (1999) showed a correlation in the evolution of IL-6 and creatine kinase. In addition, the circulating post exercise changes in IL-6 mainly correspond to the increase in the net release of IL-6 by muscle (Bernecker *et al*., 2013) and depend on the duration, intensity and mode of the exercise (Fischer, 2006; Febbraio and Pedersen, 2002; Scott *et al*., 2011).

In our study, EIB-mediated inflammation was thought to play an important role in increasing plasma levels of IL-6 observed immediately after exercise in EIB (+) subjects.

Two Hours after the Stress Test: In EIB (+) subjects, significant increases of 22.96% leukocytes, 55.87% granulocytes, 55.86% neutrophils, 54.54% eosinophils and 48.93% basophils were observed at 2 hours after the effort compared to the values obtained at pre-effort, whereas in EIB (-) subjects, a significant decrease of 29,23% lymphocytes was recorded at 2 hours after effort compared to the average value obtained before exercise, while the mean leukocyte concentrations observed at 2 hours after exercise returned to baseline (Table 4). These results revealed leukocytosis, granulocytosis, neutrophilia, eosinophilia and basophilia in the EIB (+) group and lymphopenia in the EIB (-) group. In the EIB (+) group, the late increase in leukocytes observed at 2 hours after physical exertion is mainly due to circulating neutrophils (McCarthy *et al*., 1991; Natale *et al*., 2003; Rowbottom and Green., 2000). Granulocytes, neutrophils, eosinophils and basophils continued to increase for up to 2 hours after physical exertion in EIB (+) subjects. These results could also be explained by EIB-induced inflammation in response to physical exertion. In contrast, in EIB (-) subjects, a significant decrease in lymphocytes or lymphopenia was induced by cortisol (Bishop, 2006). Lymphopenia was observed at 2 hours after exercise and showed a decrease in lymphocyte function in EIB (-) runners.

Thus, it has been suggested that the temporal decline in the function of immune variables creates a "niche" favorable to a decline in the protection of the body known as the "open window", during which viruses, bacteria and air pollutants can become established, increasing the risk of developing an infection (Pedersen and Bruunsgaard, 1995; Gleeson, 2013; Walsh *et al*., 2011). The significant decrease (29.23%) in the number of lymphocytes observed at 2 hours after physical effort in EIB (-) runners would be in agreement with the "open window" theory. Our results corroborate those of Kakanis *et al.* (2010), Sellami *et al.* (2018), Borges *et al.* (2018) and Fortunato *et al* (2018). A comparison between the plasma concentrations of IL-6 recorded at 2 h after exercise and those obtained before exercise did not show a significant difference in the athletes of either group (Table 4). In EIB (+) athletes, a return to baseline plasma levels of IL-6 was observed.

This result is consistent with previous studies (Fischer, 2006; Leggate *et al*., 2010; Pedersen and Febbraio, 2012). In this study, the results showed that inflammatory cells and IL-6 were mobilized according to the susceptibility of the subjects to bronchospasm in response to exercise. It should be noted that before effort, the measured inflammatory cell and IL-6 concentrations showed no significant difference ($p > 0.05$) between the EIB (+) and EIB (-) groups (Table 2). Similarly, a comparison of the anthropometric parameters, the duration of the exercise test and the maximum oxygen consumption did not show a significant difference ($p > 0.05$) (Table 1). Thus, what is the mechanism to explain this divergence of immune responses given that the athletes in both groups [EIB (+) and EIB (-)] were subjected to the same exercise test? Notably, physical exercise has an anti-inflammatory effect (Gleeson *et al*., 2011), and physical activity seems to attenuate the inflammatory response favoring an anti-inflammatory environment (Gleeson *et al*., 2011; Ahmed *et al*., 2012; Radom-Aizik *et al*., 2009).

Therefore, the results of this study suggest that the inflammatory state observed in the athletes of both groups is due to different inflammatory processes between the anti-inflammatory actions of exercise and those of inflammatory cells invoked by bronchospasm. The results of this study showed that in a crisis situation, the immune system of athletes sensitive to EIB is strongly solicited.

Conclusion

In this study, circulating total granulocytes, neutrophils, eosinophils, basophils and IL-6 levels were significantly increased in EIB (+) athletes at the end of the effort, whereas in EIB (-) athletes only leukocytes and systemic lymphocytes were significantly increased at the end of physical exertion. The occurrence of exercise-induced bronchospasm mobilizes a significant increase in inflammatory cells and interleukin-6. Therefore, the immune system of athletes sensitive to bronchospasm is strongly solicited.

REFERENCES

Aggarwal, B. and Mulgirigama, A, B. N. 2018. Exercise-induced bronchoconstriction: prevalence, pathophysiology, patient impact, diagnosis and management. *NPJ Prim Care Respir Med.*, *28*(1), 31.

Ahmed, HM., Blaha, MJ., Nasir, K. and Rivera, JJ, B. R. 2012. Effects of physical activity on cardiovascular disease. *Am J Cardiol.*, *109*(2), 288–295.

Becerril-Ángeles, M., Vargas, M., Hernández-Pérez, L., Rivera-Istepan, NJ., Pérez-Hidalgo, R., Ortega-González, AG., Rubio-Domínguez, S., Rodríguez-Gutiérrez, MC., Gaxiola-Cortés, R., Dosal-Ulloa, R. and Gochicoa-Rangel, L. 2017. Prevalence and Characteristics of Exercise-Induced Bronchoconstriction in High School and College Athletes at 2,240 m Altitude. *Rev Invest Clin.*, *69*(1), 20–27.

Bernecker, C., Scherr, J., Schinner, S., Braun, S. and Scherbaum, WA, H. M. 2013. Evidence for an exercise induced increase of TNF-α and IL-6 in marathon runners. *Scan J Med Sci Sports.*, *23*, 207–214.

Bishop, N.C. 2006. Acute Exercise and Aquired Immune Functione. In E. In: Gleeson M. (Ed.), *Immune Function in Sport and Exercise* (Churchill, pp. 91–113). Edinburgh, UK: Advances in Sport and Exercise Science Series.

Bonini, M, S. W. 2018. Exercise-Induced Bronchoconstriction: Background, Prevalence, and Sport Considerations. *Immunol Allergy Clin North Am.*, *38*(2), 205–214.

Bonini, M. and Silvers, W. 2018. Exercise-Induced Bronchoconstriction: Background, Prevalence, and Sport Considerations. *Immunology and Allergy Clinics of North America*. https://doi.org/https://doi.org/https://doi.org/10.1016/j.iac.2018.01.007.

Borges L, Dermargos A, Gray S, Barros Silva MB, Santos V, Pithon-Curi, TC. and Gorjão R, H. E. 2018. Neutrophil Migration and Adhesion Molecule Expression after Acute High-Intensity Street Dance Exercise. *J Immunol Res*, *2018*, 1684013.

Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, TL. and MacLean, DA. P. B. 1997. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *J Physiol.*, *499*(3), 833–841.

Burnett, DM., Burns, S., Merritt, S. and Wick, J, S. M. 2016. Prevalence of Exercise-Induced Bronchoconstriction

Measured by Standardized Testing in Healthy College Athletes. *Respir Care.*, *61*(5), 57–6.

Bussotti, M., Di Marco, S. and Marchese, G. 2014. Respiratory disorders in endurance athletes–how much do they really have to endure? *Open Access J Sports Med.*, *5*, 47–63.

Caggiano, S., Cutrera, R., Di, M., et T. A., 2017. Exercise-Induced Bronchospasm and Allergy. *Front Pediatr.*, *5*, 131–138.

Cavaleiro Rufo, J., Paciência, I., Silva, D., Martins, C., Madureira, J., Oliveira Fernandes, E., Padrão, P., Moreira, P. and Delgado L, M. A. 2018. Swimming pool exposure is associated with autonomic changes and increased airway reactivity to a beta-2 agonist in school aged children: A cross-sectional survey. *PLoS One*, *13*(3), e0193848.

Colbey, C., Cox, AJ., Pyne, DB., Zhang, P. and Cripps, AW W. N. 2018. Upper Respiratory Symptoms, Gut Health and Mucosal Immunity in Athletes. *Sports Med*, *48*(1), 65–77.

Croisier, JL., Camus, G., Venneman, I., Deby-Dupont, G., Juchmès-Ferir, A., Lamy, M., *et al*., 1999. Effects of training on exercise-induced muscle damage and interleukin 6 production. *Muscle Nerve*, *22*(2), 208–212.

Cruzat, VF. and Krause M, N. P. 2014. Amino acid supplementation and impact on immune function in the context of exercise. *J IntSoc Sports Nutr.*, *11*(1), 61.

Del Giacco, SR., Firinu, D. and Bjermer L, C. K. 2015. Exercise and asthma: an overview. *Eur Clin Respir J*, *2*, 279–284.

Febbraio, MA, P. B. 2002. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *The FASEB J*, *16*, 1335–1347.

Fischer, CP. 2006. Interleukin-6 in acute exercise and training: what is the biological relevance? *Exerc Immunol Rev*., 12: 6-33.

Florent Nsompi, A. T. and Folly Messan, M. M. L. 2018. Prevalence of exercise-induced bronchospasm among athletes in the hot and humid climate of brazzaville, congo. *International Journal of Development Research*, *8*(12), 24473–24479.

Fortunato, AK., Pontes, WM., De Souza, DMS., Prazeres, JSF., Marcucci-Barbosa, LS., Santos, JMM., Veira, ÉLM., Bearzoti, E., Pinto, KMC. and Talvani, A, D. S. A. 2018. Strength Training Session Induces Important Changes on Physiological, Immunological, and Inflammatory Biomarkers. *J Immunol Res.*, 9675216.

Gleeson, M. 2013. Exercise, nutrition and immunity. In: P.C. Calder and P. Yaqoob (eds.), Diet, Immunity and Inflammation. *Cambridge: Woodhead Publishing*, 652–685.

Gleeson, M., Bishop, NC., Stensel, DJ., Lindley, MR, SS and Mastana, N. M. 2011. Les effets anti-inflammatoires de l'exercice: mécanismes et implications pour la prévention et le traitement de la maladie. *Nat Rev Immunol.*, *11*(9), 607–615.

Hallstrand, TS., Altemeier, WA. and Aitken, MI, H. W. 2013. Role of cells and mediators in exercise-induced bronchoconstriction. *Immunol Allergy Clin North Am.*, *33*(3), 313–317.

Hallstrand, TS., Moody, MW., Wurfel, MM., Schwartz, LB. and Henderson, WR Jr, A. M. 2005. Inflammatory basis of exercise-induced bronchoconstriction. *Am J Respir Crit Care Med.*, *172*, 679–86.

Hansen, J.B. and Wilsgard L., O. B. 1991. Biphasic changes in leukocytes induced by strenuous exercise. *European Journal of Applied Physiolog*, *62*, 157–161.

Holzer K, D. J. 2006. Exercise induced bronchoconstriction in elite athletes: measuring the fall. *Thorax*, *61*, 94–96.

https://doi.org/https://doi.org/https://doi.org/10.1016/j.scispo.2011.08.001.

Joshi S, Powell T, Watkins WJ, Drayton M, Williams EM, K. S. (2013). Exercise-induced bronchoconstriction in school-aged children who had chronic lung disease in infancy. *J Pediatr*, *162*(4), 813–8.

Kakanis, MW., Peake, J., Brenu, EW., Simmonds, M., Gray, B., Hooper, SL. and M.-G. S. 2010. The open window of susceptibility to infection after acute exercise in healthy young male elite athletes. *Exerc Immunol Rev*, *16*, 119–137.

Karalaki, M., Fili, S. and Philippou, A. K. M. 2009. Muscle regeneration: cellular and molecular events. *In Vivo*, *23*(5), 779–796.

Kippelen, P., Larsson, J., Anderson, SD., Brannan, JD. And Dahlén B, D. S. 2010. Effect of sodium cromoglycate on mast cell mediators during hyperpnea in athletes. *Med Sci Sports Exerc*, *42*, 1853–60.

Kurowski, M., Jurczyk, J., Moskwa, S., Jarzębska, M. and Krysztofiak, H, K. M. 2018. Winter ambient training conditions are associated with increased bronchial hyperreactivity and with shifts in serum innate immunity proteins in young competitive speed skaters. *Arch Med Sci*, *14*(1), 60–68.

Larsson, K., Ohlsén, P., Larsson, L., Malmberg, P. and Rydström PO, U. H. 1993. High prevalence of asthma in cross country skiers. *BMJ*, *307*(6915), 1326–1329.

Leggate, M., Nowell, MA., Jones, SA. and Nimmo, MA. 2010. The response of interleukin-6 and soluble interleukin-6 receptor isoforms following intermittent high intensity and continuous moderate intensity cycling. *Cell Stress and Chaperones*, *15*, 827–833.

Mahmoud, AM., Mohsen, G., Abdolhamid, H. and Saeed, S. M. N. 2013. The effect of high intensity interval exercise in high / low temperatures on exercise-induced bronchoconstriction (EIB) in training adolescent males. *Tanaffos*, *12*(3), 29–43.

McCarthy, D.A., Grant, M., Marbut, M., Watling, M., Wade, A.J., Macdonald, I., Nicholson, S. and Melsom, R.D., P. J. . 1991. Brief exercise induces an immediate and a delayed leukocytosis. *British Journal of Sports Medicine*, *25*(4), 191–195.

Meckel, Y., Eliakim, A., Seraev, M., Zaldivar, F., Cooper, DM. and Sagiv, M, N. D. 2009. The effect of a brief sprint interval exercise on growth factors and inflammatory mediators. *J Strength Cond Res*, *23*, 225–230.

Messan, F., Dansou, P., Lawani, M., Falola, J. and Gouthon, P. 2011. Prévalence du bronchospasme induit par l'exercice chez des basketteurs amateurs en climat chaud et humide. *Rev. CAMES-Série A*, *12*(1), 28–33.

Messan, F., Tito, A, Gouthon, P, Nouatin, K., Nigan, I., Blagbo, A. and Lounana J, M. J. 2017. Comparison of catecholamine values before and after Exercise-Induced bronchospasm in professional cyclists. *Tanaffos*, *16*(2), 136–143.

Mohammadreza Modaresi, Roya Kelishadi, Akram Amiri, Akbar Amiri, Zahra Aryan and Babak Amra, J. al F. 2015. The association of particulate matter air pollution with exercice-induced bronchospasm. *European Respiratory Journal*, *46*(59), PA4504.

Natale, V.M., Brenner, I.K., Moldoveanu, A.I., Vasiliou, P. and Shek, P., S. R. 2003. Effects of three different types of exercise on blood leukocyte count during and following exercise. *Sao Paulo Medical Journal*, *121*(1), 9–14.

Nieman, D., Henson, D., Gojanovich, G., Vevis, JM., Dumkel, C., Utter, A., Murphy, A., Pearcel, S. and McAnulty, S, M. L. (2007). Immune changes: 2h of continuous vs intermittent cycling. *Int J Sports Med*, *28*, 1–6.

Ostrowski, K., Rohde, T., Asp, S. and Schjerling, P. P. B. 1999. Pro- and ant-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol (Lond)*, *515*, 287–291.

Ouattara, S., Balayssac-Siransy, A. E., Konaté, A., Tuo, N., Keita, M., Dah, C. and Bogui, P. 2012. Bronchospasme induit par l'exercice chez des sportifs de compétition en milieu tropical humide. *Science and Sports*, *27*, 1–8.

Pedersen BK, F. M. 2012. Muscle, exercise and obesity: skeletal muscle as secretory organ. *Nat Rev Endocrinol*, *8*, 457–465.

Pedersen, B. B. H. 1995. How physical exercise influences the establishment of infection. *Sports Med*, *19*, 393–400.

Perry, C., Pick, M., Bdolach, N., Hazan-Halevi, I., Kay, S., Berr, I., Reches, A. and Harishanu, Y, G. D. 2013. Endurance exercise diverts the balance between Th17 cells and regulatory Tcells. *PLoS One*, *8*(10), e74722.

Radom-Aizik, S., Zaldivar, F. and Jr, Leu, SY, C. D. 2009. Brief bout of exercise alters gene expression in peripheral blood mononuclear cells of early- and late-pubertal males. *Pediatr Res*, *65*(4), 447–452.

Robson-Ansley, P., Howatson, G., Tallent, J., Mitcheson, K., Walshe, I. and Toms, C, *et al*., 2012. Prevalence of allergy and upper respiratory tract symptoms in runners of the London marathon. *Med Sci Sports Exerc*, *44*(6), 999–1004.

Rowbottom, D.G., G. K. 2000. Acute exercise effects on the immune system. *Medicine & Science in Sports & Exercise*, *32*(7), S396–S405.

Rundell, KW, S.-C. M. 2013. Air quality and exercise-induced bronchoconstriction in elite athletes. *Immunol Allergy Clin North Am*, *33*(3), 409–421.

Rundell, KW. and Smoliga JM, B. V. 2018. Exercise-induced bronchocontriction and the air we breathe. *Immunol Allergy Clin North Am*, *38*(2), 183–204.

Rundell, KW., Anderson, SD., Sue-Chu, M. and Bougault, V, B. L. 2015. Air quality and temperature effects on exercise-induced bronchoconstriction. *Compr Physiol*, *5*(2), 579–610.

Scherr, J., Braun, S., Schuster, T., Hartmann, C., Moehlenkamp, S., Wolfarth, B. and Pressler, A, H. M. 2011. 72-h kinetics of highsensitive troponin T and inflammatory markers after marathon. *Med Sci Sports Exerc*, *43*, 1819–1827.

Schild, G., Eichner, G., Beiter, T., Zügel, M., Krumholz-Wagner, I. and Hudemann, J, *et al*., 2016. Effects of acute endurance exercise on plasma protein profiles of endurance-training and untraining individuals over time. *Hindawi Publishing Corporation Mediators of Inflammation Volume*, *Article ID*, 11 pages. http//dx.doi.org/10.1155/2016/4851935.

Scott, JPR., Sale, C., Greeves, JP., Casey, A. and Dutton, J, F. W. 2011. Effect of Exercise Intensity on the Cytokine Response to an Acute Bout of Running. *Med Sci Sports Exerc*, *43*, 2297–2306.

Sellami, M., Gasmi, M., Denham, J., Hayes, LD., Stratton, D. and Padulo, J, B. N. 2018. Effects of Acute and Chronic Exercise on Immunological Parameters in the Elderly Aged: Can Physical Activity Counteract the Effects of Aging? *Front Immunol*, *9*, 2187.

Smith, LR. and Meyer, G, L. R. 2013. Systems analysis of biological networks in skeletal muscle function. *Wiley Interdiscip Rev Syst Biol Med*, *5*(1), 55–71.

Toigo, M, B. U. 2006. New fundamental resistance exercise determinants of molecular and cellular muscle adaptations. *Eur J Appl Physiol*, *97*(6), 643–663.

Walsh, N.P., Gleeson, M., Shephard, R.J., Gleeson, M., Woods, J.A., Bishop, N.C., Fleshner, M., Green, C., Pedersen, B.K., Hoffman-Goetz, L., *et al*., 2011. Position statement. Part one: Immune function and exercise. *Exerc. Immunol. Rev*, *17*, 6 – 63.

Weiler, JM., Brannan, JD., Randolph, CC., Hallstrand, TS., Parsons, J., Silvers, W., Storms, W., Zeiger, J., Bernstein, DI., Blessing-Moore, J., Greenhawt, M., Khan, D., Lang, D, Nicklas RA, Oppenheimer J, Portnoy JM, Schuller DE. and Tilles SA, W. D. 2016. Exercise-induced bronchoconstriction update-2016. *J Allergy Clin Immunol*, *138*(5), 1292–1295.

Zweetsloot, KA., John, C., Lawrence, M. and Battista, RA., S. R. 2014. High-intensity interval training induces a modest systemic inflammatory response in active, young men. *Journal of Inflammation Research*, *7*, 9–17.

****