

Changes in RANTES and β -Thromboglobulin after Intensive Exercise in Patients with Allergic Asthma

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Key Words

Asthma • Exercise-induced bronchoconstriction • RANTES • β -Thromboglobulin • Airway inflammation

Abstract

Background: There is increasing evidence that exercise-induced bronchoconstriction (EIB) is associated with eosinophilic airway inflammation. In the pathogenesis of EIB the role of chemokines – responsible for promoting the migration and activation of inflammatory cells – as well as blood platelets, a potential source of those chemokines, remains unclear. **Methods:** The study was conducted in a group of 19 asthmatics (11 with EIB, 8 without EIB) and 8 healthy volunteers. Changes in the plasma concentrations of RANTES and β -thromboglobulin (β -TG) induced by intensive exercise were determined. Moreover, the possible correlation of these measurements with the results of other tests used in the diagnosis of asthma as well as laboratory tests commonly associated with asthma were investigated. **Results:** A comparison of the concentrations of β -TG in all groups studied at rest did not reveal any significant differences. In all groups studied, 30 min after exercise elevated β -TG concentrations were observed; the most significant increase was revealed in asthmatics with EIB. The baseline concentrations of RANTES before exercise in both groups of asthmatics were signifi-

cantly higher in comparison to the group of healthy volunteers. After exercise, in the group of patients with EIB, a significant increase in RANTES concentrations was observed. These changes correlated with an increase in other markers of airway inflammation 24 h after exercise. **Conclusions:** We suggest that platelet activation, resulting in elevated RANTES release, could be one of the factors responsible for the increase of airway inflammation observed in consequence of EIB in asthmatics.

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Introduction

Asthma has become recognized as a chronic inflammatory disease associated with inflammatory cell infiltration and airway epithelial damage. Eosinophils, T lymphocytes and mast cells are the key effector cells in asthma [1]. However, platelets, which are an important source of many biologically active mediators, can also participate in this allergic inflammatory process.

Many investigations into the role of platelets in asthma have focused on their aggregation, release of platelet factor-4 (PF-4) and β -thromboglobulin (β -TG), and their accumulation in lungs. Platelets have been found to accumulate in the pulmonary microvasculature and been

detected in the bronchoalveolar lavage fluid of patients undergoing bronchial provocation with allergens [2]. Some reports concerning the release of chemokines, such as PF-4 and RANTES, from platelets have also been published [3]. Kameyoshi et al. [4] demonstrated that agonist-mediated activation of platelets is augmented in asthmatics, resulting in enhanced secretion of chemokines such as RANTES. These chemokines, released from platelets after stimulation, have been implicated in the allergic inflammation of asthma by promoting the migration and activation of inflammatory cells, including eosinophils [5].

Eosinophils initiate tissue damage by the release of cytotoxic substances like major basic protein, eosinophil cationic protein (ECP), eosinophil peroxidase, and the autocrine production of chemokines which cause a self-sustaining inflammatory process and chronic disease [6]. Eosinophils play a crucial role in inflammation in asthma [1], but their role in exercise-induced bronchoconstriction (EIB) still remains to be elucidated.

Yoshikawa et al. [7] have revealed that there is a relationship between the severity of EIB and the eosinophil count in induced sputum as well as in serum ECP levels in adult asthmatic patients. This suggests that the presence of sputum eosinophilia predicts the airway responsiveness induced by an exercise challenge. Eosinophilic inflammation is also augmented by postexercise bronchoconstriction, which in turn is stimulated by many mediators released mainly by mast cells [8]. We observed the upregulation of airway inflammation, expressed as an increase in exhaled nitric oxide (F_{ENO}) levels and in bronchial hyperresponsiveness to histamine after postexercise bronchoconstriction in allergic asthma patients [9].

A number of reports have been published on the effect of exercise on platelet activity [10]. During exercise-associated stimulation, platelets can release many chemotactic substances and activation markers (e.g. β -TG and PF-4) [10].

In the present study we have decided to investigate the role of platelets and RANTES, released during their activation, in the pathogenesis of EIB in asthmatic patients. We have tried to establish whether there is a difference with regard to platelet function and platelet RANTES release between asthmatics with EIB or without EIB and healthy persons. We determined changes in plasma concentrations of RANTES and β -TG following intensive exercise in order to establish the possible correlation of these measurements with the parameters of airway inflammation and their changes after exercise.

Methods

Subjects

The study involved a group of 19 patients with mild allergic asthma (11 with EIB, 8 without EIB). Asthma was diagnosed according to the criteria recommended by the GINA [11]. The presence of an allergy to common allergens (house dust mite, trees, weeds, grasses, cat, *Alternaria*, or *Cladosporium*) was demonstrated in all patients by positive skin-prick tests. The characteristics of the patients studied are presented in table 1. All patients had been in a stable condition, free from respiratory tract infections and acute exacerbations during the past 4 weeks. Patients with other factors which could influence F_{ENO} levels (except for asthma, features of atopy, or allergic rhinitis) were excluded from the study. Before the beginning of this study, patients were allowed to take short-acting β_2 -agonists. Asthmatic patients, who had been treated with inhaled steroids in the preceding 3 months, were excluded from the study.

The control group included 8 healthy volunteers. F_{ENO} , flow/volume spirometry, and prick tests with common inhaled allergens were performed in all healthy persons. All of them were free of chronic or recurrent respiratory symptoms with no past history of significant pulmonary disease. They were non-atopic and had $FEV_1 > 80\%$ predicted. They were free of respiratory tract infection within 2 months prior to the study as well as from other significant illnesses known to affect F_{ENO} measurements.

All asthmatic patients studied and healthy volunteers were non-smokers and during the last year had not been passive smokers. Total IgE, serum ECP, peripheral blood eosinophilia and platelet count were determined in all asthmatic patients and healthy volunteers. In all subjects studied an exercise test on a bicycle ergometer was performed. 24 h after exercise, measurements of serum ECP, peripheral blood eosinophilia, platelet count and F_{ENO} were determined and a bronchial provocation test with histamine was performed.

The study protocol was approved by the Ethics of Research Committee of the Medical University of Białystok (Agreement No. R-I-003/80/2006). Informed consent was obtained from every patient entered into the study.

Measurements

The baseline spirometry was performed using a MasterScreen Pneumo PC spirometer (Jaeger, Höchberg, Germany). Spirometry was performed according to ATS standards [12]. FEV_1 (forced expiratory volume in 1 s) was evaluated. Before the examination, the patients did not take any medications that could change spirometry results.

A non-specific bronchial provocation test with histamine (BPT) was carried out according to the method described by Ryan et al. [13]. Provocation was performed using a De Villbiss nebulizer 646 (Viasys Healthcare GmbH, Höchberg, Germany) at an air pressure of 0.15 MPa linked to a Rosenthal-French dosimeter (Baltimore, Md., USA). The results were presented as PC_{20} FEV_1 – the concentration of histamine which causes a decrease in FEV_1 of exactly 20% in comparison to initial values.

An exercise test was performed on a bicycle ergometer for 9 min with a fixed workload adjusted to increase the heart rate to 85% of the maximum predicted for the age of each patient [14]. Serial FEV_1 measurements were made 5 min before exercise and at 5, 10, 15, 20, 25, 30, 60 min, and 24 h after exercise. Those pa-

Table 1. Characteristics of study subjects and healthy volunteers

Characteristics	Patients with EIB	Patients without EIB	Differences between groups of asthma patients, p value	Healthy volunteers
Patients, n	11	8		8
Female/male	7/4	5/3		4/4
Age, years	33.45 ± 7.80	33.25 ± 7.04	0.95	29.87 ± 7.05
Duration of symptoms, years	4.2 ± 3.5	3.6 ± 2.3	0.67	
Baseline FEV ₁ , % predicted	86.72 ± 9.10	92.87 ± 5.96	0.11	98.37 ± 4.45 ^{a, b}
logPC ₂₀ histamine FEV ₁ , mg/ml	-0.05 ± 0.51	-0.04 ± 0.71	0.98	
Serum ECP, µg/l	14.56 ± 2.51	11.11 ± 2.11	0.005	2.88 ± 0.84 ^{a, b}
Serum total IgE, kU/l	270 ± 128	191 ± 74	0.13	39 ± 12 ^{a, b}
Blood eosinophil count, cells/mm ³	262 ± 142	189 ± 97	0.18	68 ± 37 ^{a, b}
Platelets, cells/µl	223 ± 42	214 ± 34	0.87	201 ± 53
Baseline F _{ENO} , ppB	84 ± 49	60 ± 36	0.27	15 ± 5 ^{a, b}
Baseline RANTES, pg/ml	7,089.55 ± 2,979.85	7,173.50 ± 2,280.78	0.94	4,700.38 ± 1,373.84 ^{a, b}
Baseline β-TG, IU/ml	53.77 ± 14.47	53.22 ± 13.09	0.93	51.33 ± 11.22

Data are presented as medians (ranges).

FEV₁ = Forced expiratory volume in 1 s; PC₂₀ histamine FEV₁ = provocative concentration of histamine that caused a 20% fall in FEV₁; ECP = eosinophil cationic protein; RANTES = regulated upon activation normal T cell expressed and presumably secreted (CCL5); β-TG = β-thromboglobulin.

^a Values significantly different from patients with EIB, *p* < 0.05.

^b Values significantly different from patients without EIB, *p* < 0.05.

tients whose maximum decrease in FEV₁ was greater than 15% from baseline were considered to have EIB. The blood samples were collected before, 30 min after, and 24 h after the exercise test.

F_{ENO} was measured by the chemiluminescence technique, using a Sievers 280i NO Analyzer (Boulder, Colo., USA). The measurements were performed at an expiratory flow of 50 ml/s according to ATS recommendations for online measurement of F_{ENO} in adults [15].

Serum total IgE and ECP concentrations were measured using ImmunoCAP™ Technology (Pharmacia Diagnostics, Uppsala, Sweden). Platelet count and peripheral blood eosinophilia were determined using an automatic hematological analyzer AD-VIA-120 (Bayer AG, Leverkusen, Germany).

Plasma samples were obtained using citrate-theophylline-adenosine-dipyridamole (CTAD) anticoagulation for evaluation of β-TG (BD Vacutainer CTAD 2.7-ml collection tubes; BD Diagnostics, Plymouth, UK) and using citrate as an anticoagulant for evaluation RANTES (precooled BD Vacutainer 9NC, 0.129 M, 4.5-ml collection tubes; BD Diagnostics, UK). CTAD anticoagulated blood samples were incubated on ice for 30 min and then centrifuged at 2,500 *g* for 30 min at 4°C. The supernatants were aliquoted and stored at -80°C until tested. Citrate anticoagulated blood samples were centrifuged at 2,500 *g* for 20 min at 4°C, within 30 min of collection. Plasma supernatants were aliquoted and stored at -80°C until tested.

The plasma concentrations of the RANTES (R&D Systems, Wiesbaden-Nordenstadt, Germany; detection limit 2.0 pg/ml) and β-TG (Diagnostica Stago, Asnières, France; detection limit 5.0 IU/ml) were determined using enzyme-linked immunosor-

bent assay. All measurements were performed in a blinded fashion. All samples were run in duplicate.

Analysis

Statistical significance was analyzed by using analysis of variance (ANOVA) followed by Bonferroni's post-hoc *t* test to determine statistical differences. All values were expressed as means ± SD; *p* values <0.05 were considered significant. PC₂₀ values were logarithmically transformed for analysis. The relationship between the parameters studied was assayed by correlation. Pearson's linear correlation coefficient was used.

Results

The characteristics of asthmatic patients and healthy volunteers are presented in table 1. In the group of asthmatics studied, 11 patients had a positive and 8 had a negative exercise test. In none of the healthy volunteers were spirometric indices worse after exercise. In both groups of asthmatics there were no statistically significant differences in baseline spirometric indices, non-specific bronchial hyperreactivity, total IgE, blood eosinophil count, or the levels of F_{ENO}, however in the group of patients with positive exercise tests, statistically significantly higher ECP levels were observed.

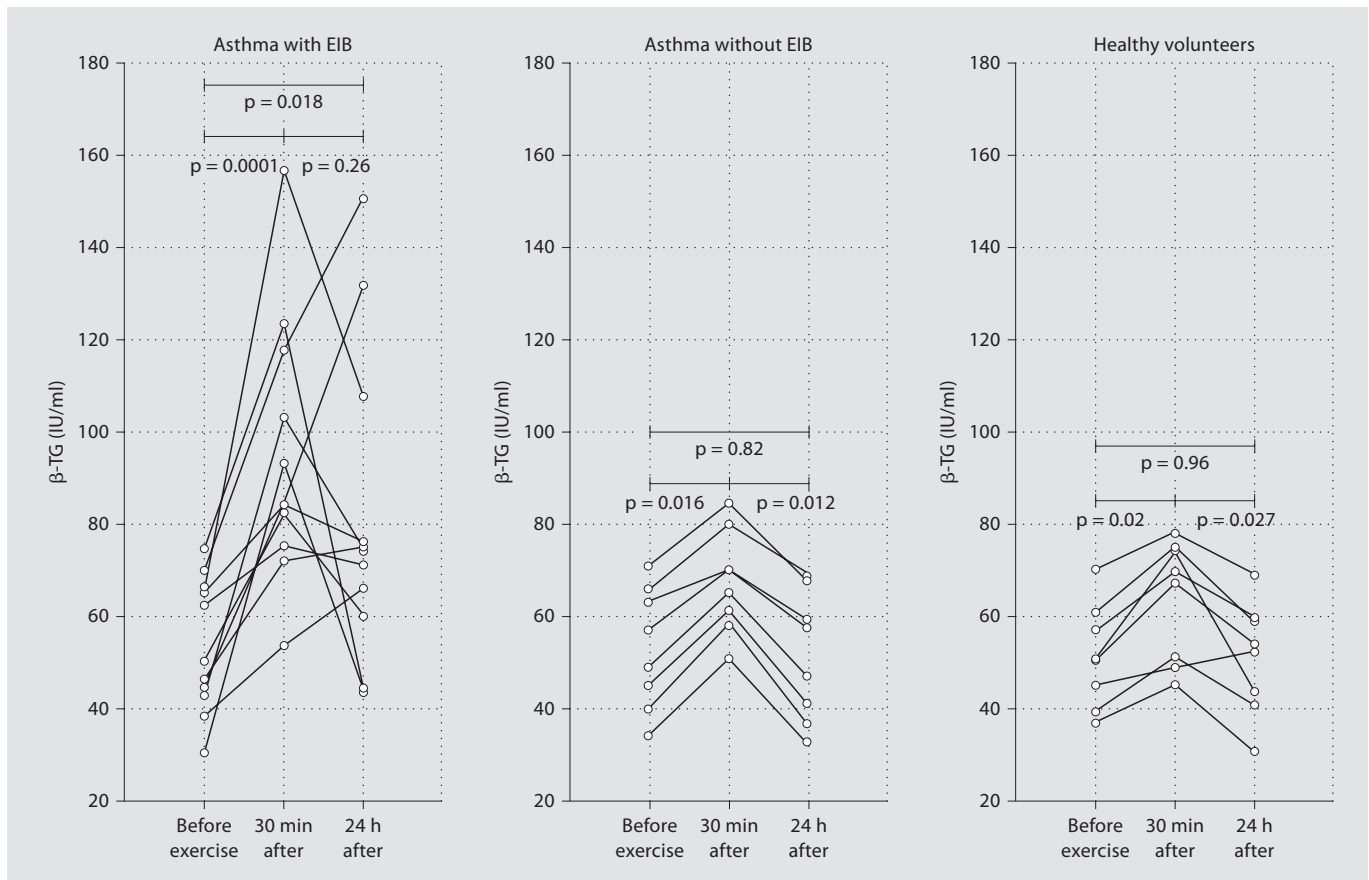


Fig. 1. Changes in the plasma concentrations of β -TG induced by intensive exercise in the groups of asthmatics studied and healthy volunteers.

Exercise-Induced Changes in Baseline Levels of the Parameters Studied

A comparison of the concentrations of β -TG in both groups of asthmatic patients and healthy volunteers at rest did not reveal any statistically significant differences (asthma with EIB: 53.77 ± 14.47 IU/ml vs. asthma without EIB: 53.22 ± 13.09 IU/ml, $p = 0.93$, vs. healthy volunteers: 51.33 ± 11.23 IU/ml, $p = 0.69$; asthma without EIB vs. healthy volunteers: $p = 0.76$). 30 min after exercise in all groups studied a statistically significant increase in the concentration of β -TG was observed, however the most elevated levels of this chemokine and the highest statistical relevance were observed in the group of asthmatics with EIB (patients with EIB: 95.07 ± 28.71 IU/ml; patients without EIB: 67.55 ± 11.1 IU/ml; healthy volunteers: 63.67 ± 13.14 IU/ml). 24 h after exercise the concentration of β -TG was significantly higher in comparison to the baseline values only in the group of patients

with EIB (patients with EIB: 88.88 ± 34.14 IU/ml; patients without EIB: 51.45 ± 11.1 IU/ml; healthy volunteers: 51.25 ± 12.12 IU/ml) (fig. 1).

The concentrations of RANTES before exercise in both groups of asthma patients were statistically significantly higher in comparison to the group of healthy volunteers (patients with EIB: $7,089.55 \pm 2,979.85$ pg/ml vs. patients without EIB: $7,173.50 \pm 2,280.78$ pg/ml, $p = 0.94$, vs. healthy volunteers: $4,700.38 \pm 1,373.84$ pg/ml, $p = 0.046$, asthma without EIB vs. healthy volunteers: $p = 0.02$). In the group of patients with EIB, a statistically significant increase in RANTES levels was observed 30 min after exercise ($9,512.73 \pm 2,817.55$ pg/ml). 24 h after exercise, the concentration of RANTES had subsequently increased ($11,779.73 \pm 2,853.72$). There were no significant changes in the group of asthma patients without EIB and healthy volunteers before and after exercise (fig. 2).

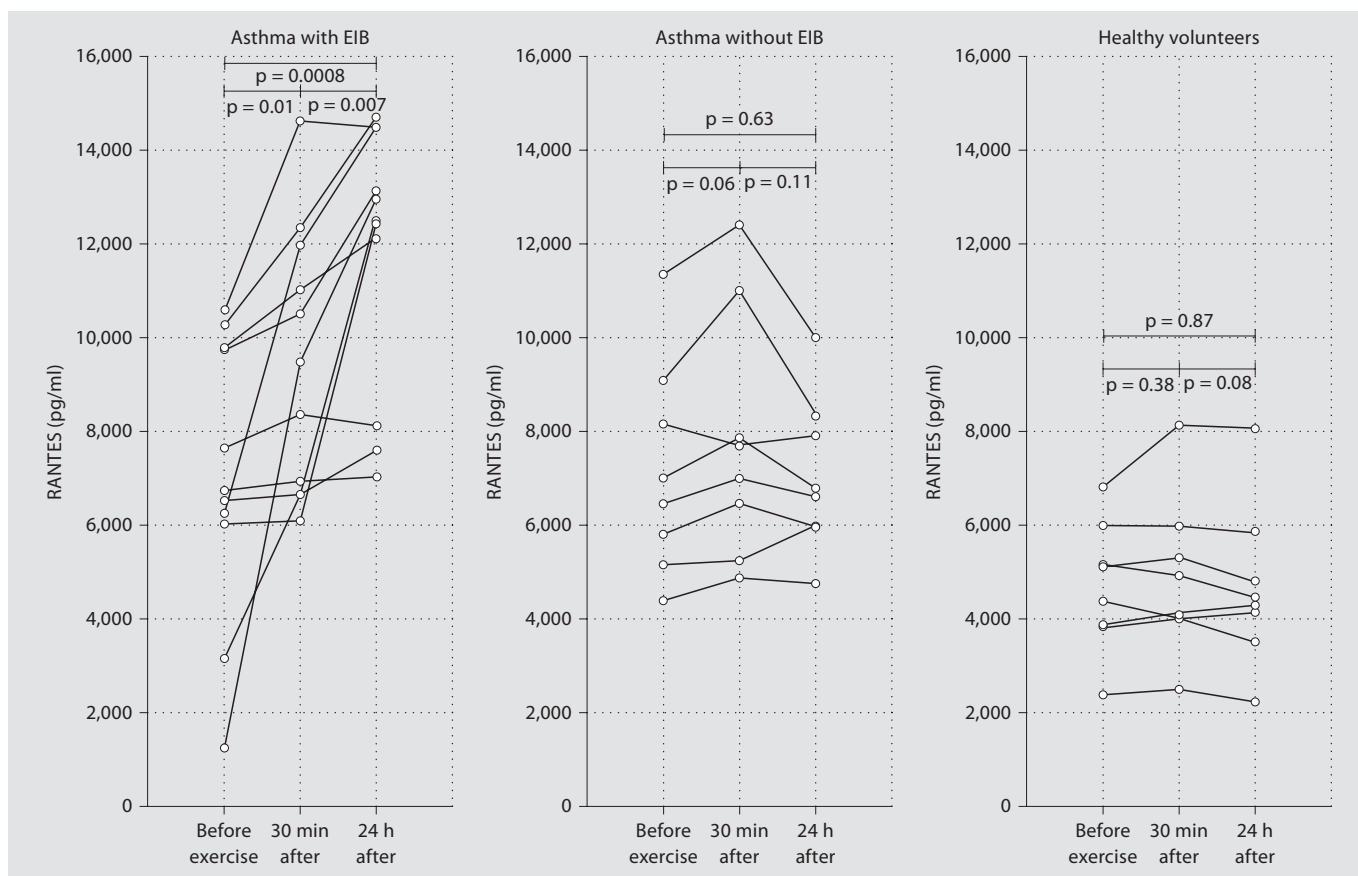


Fig. 2. Concentrations of plasma RANTES at rest, and subsequent changes during the exercise test in groups of patients with asthma and healthy volunteers.

Correlations between RANTES and β -TG and Other Parameters Studied

There were no statistically significant correlations between the baseline concentrations of β -TG or RANTES and other parameters studied in either group of asthmatic patients or the healthy volunteers.

We have discovered a statistically significant positive correlation between the increase in RANTES concentrations, observed 30 min after exercise, and serum ECP ($r = 0.68$, $p = 0.02$) or F_{ENO} ($r = 0.66$, $p = 0.02$) before exercise, and the increase in RANTES concentrations 24 h after exercise and serum ECP ($r = 0.63$, $p = 0.036$) or F_{ENO} ($r = 0.77$, $p = 0.005$) before exercise in asthmatics with EIB (fig. 3). There was no statistically significant correlation between postexercise changes in β -TG concentrations and the results of other tests performed before exercise. There were no significant correlations between the severity of EIB and the degree of change in plasma β -TG and RANTES levels induced by the exercise challenge.

Correlations between the Changes in F_{ENO} , BHR, and Serum ECP 24 h after Exercise and Other Parameters Studied

Changes in F_{ENO} , BHR, and serum ECP 24 h after exercise are presented in table 2. 24 h after the exercise test in the group of asthmatics with EIB, a statistically significant increase in F_{ENO} , serum ECP concentrations and BHR to histamine was revealed. Such changes were not observed in the group of asthmatic patients without EIB.

A statistically significant correlation between the increase in RANTES concentrations 30 min after exercise and an increase in F_{ENO} ($r = 0.53$, $p = 0.039$), an increase in ECP ($r = 0.59$, $p = 0.027$) or a decrease in $\log PC_{20} FEV_1$ ($r = -0.61$, $p = 0.045$) 24 h after exercise were revealed (fig. 4). Such correlations between the increase in RANTES concentrations 24 h after exercise and an increase in F_{ENO} ($r = 0.68$, $p = 0.019$), an increase in ECP ($r = 0.67$, $p = 0.021$) or a decrease in $\log PC_{20} FEV_1$ ($r = -0.63$,

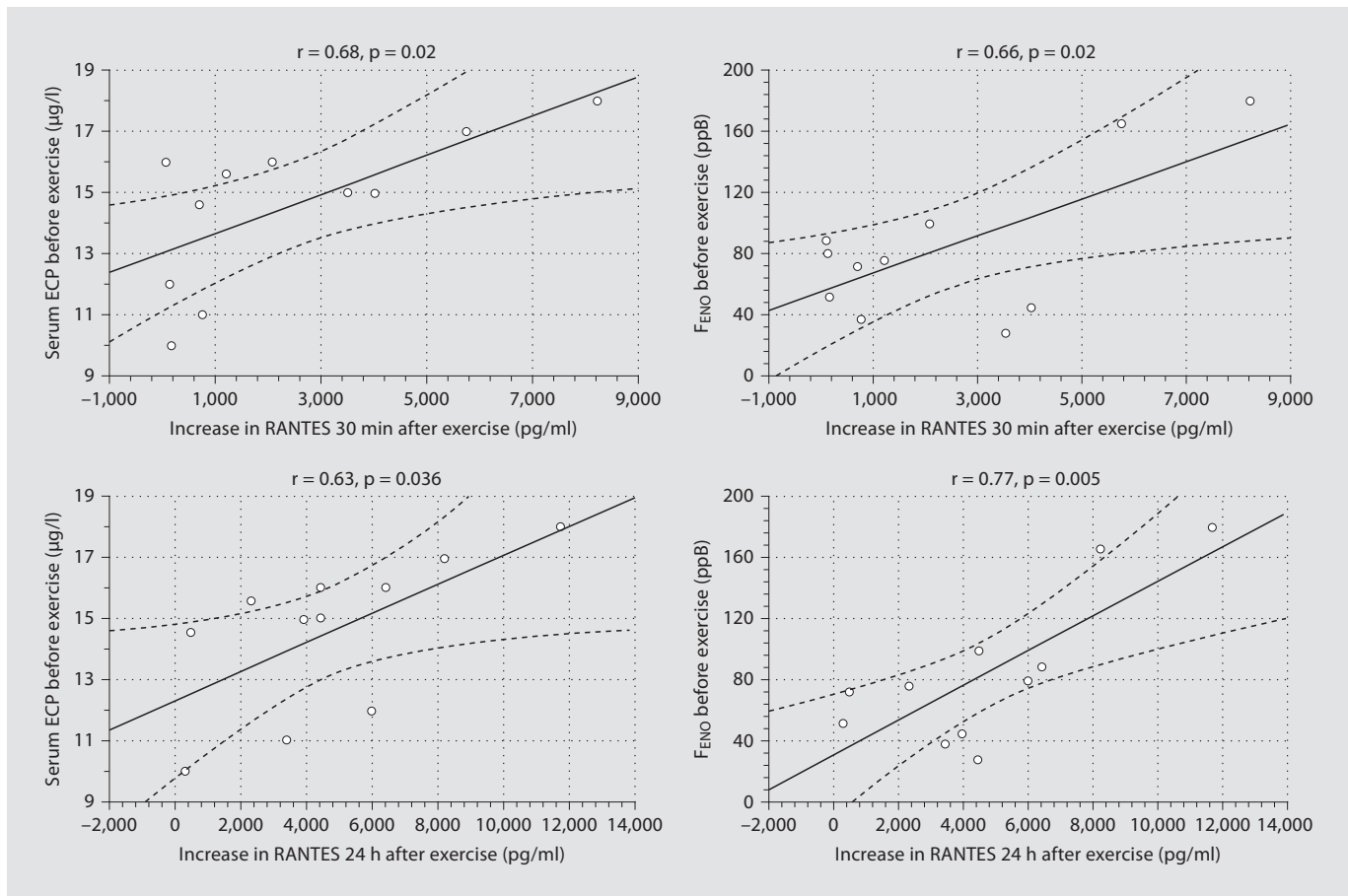


Fig. 3. Correlations between serum ECP or F_{ENO} before exercise and increase in RANTES 30 min or 24 h after exercise.

Table 2. Changes in F_{ENO} , BHR to histamine, and serum ECP 24 h after exercise in the studied groups of asthmatics and healthy volunteers

Studied groups	F_{ENO} , ppB			BHR to histamine, logPC ₂₀ FEV ₁			ECP, µg/l		
	before exercise	24 h after exercise	p	before exercise	24 h after exercise	p	before exercise	24 h after exercise	p
Patients with EIB	84.00 ± 49.15	101.18 ± 52.77	0.0005	-0.05 ± 0.51	-0.13 ± 0.58	0.03	14.56 ± 2.51	17.95 ± 3.72	0.03
Patients without EIB	60.75 ± 36.52	58.81 ± 27.30	0.47	-0.04 ± 0.71	0.04 ± 0.46	0.42	11.11 ± 2.11	12.05 ± 2.83	0.41
Healthy volunteers	15.02 ± 5.10	14.41 ± 3.80	0.51				2.88 ± 0.84	2.61 ± 0.75	0.53

F_{ENO} = Exhaled nitric oxide; PC₂₀ histamine FEV₁ = provocative concentration of histamine that caused a 20% fall in FEV₁; ECP = eosinophil cationic protein; EIB = exercise-induced bronchoconstriction.

$p = 0.035$) 24 h after exercise were also observed (fig. 5).

There was no correlation between the changes of β -TG concentrations 30 min and 24 h after exercise and other

parameters described earlier. We did not reveal statistically significant changes in blood platelet and eosinophil count during the study in either the group of patients studied or healthy controls (data not shown). No changes

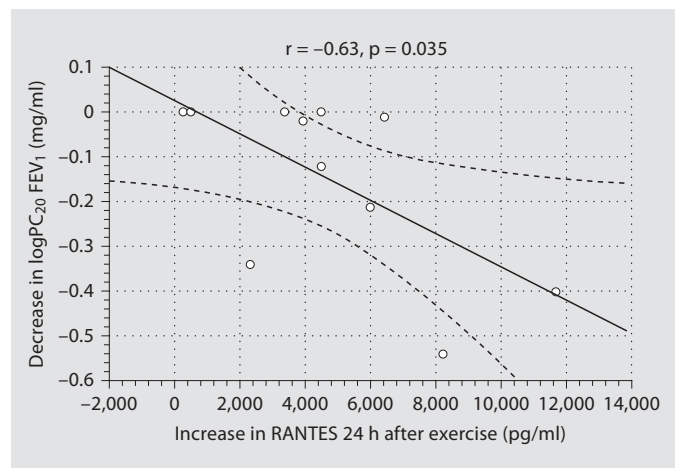
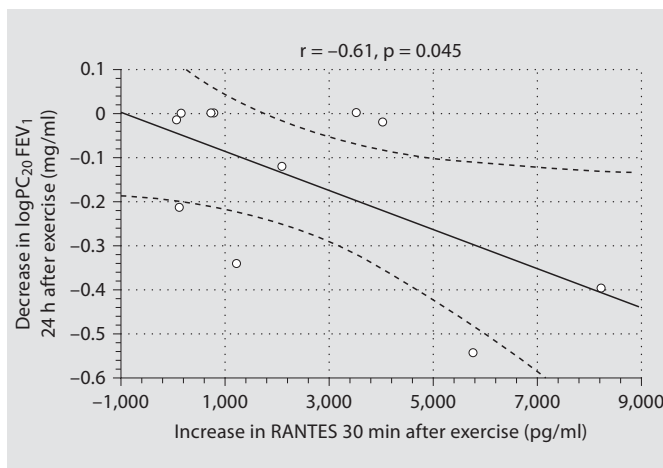
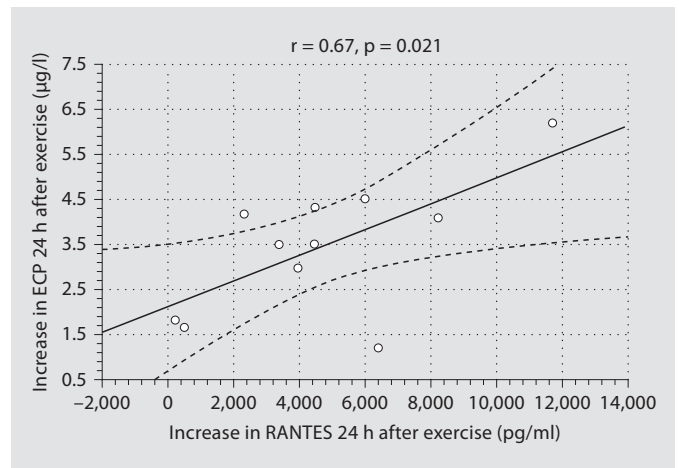
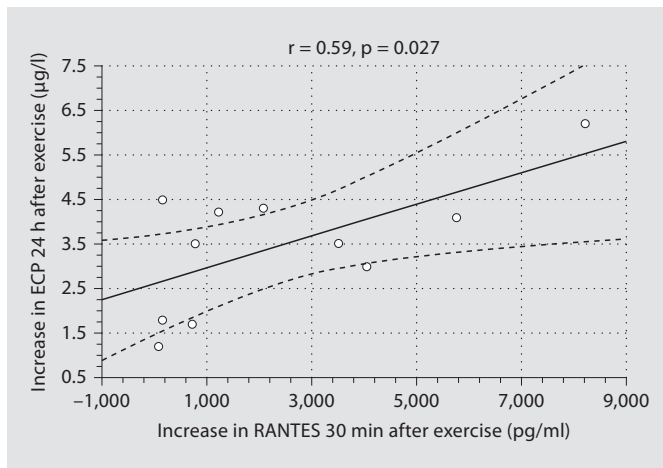
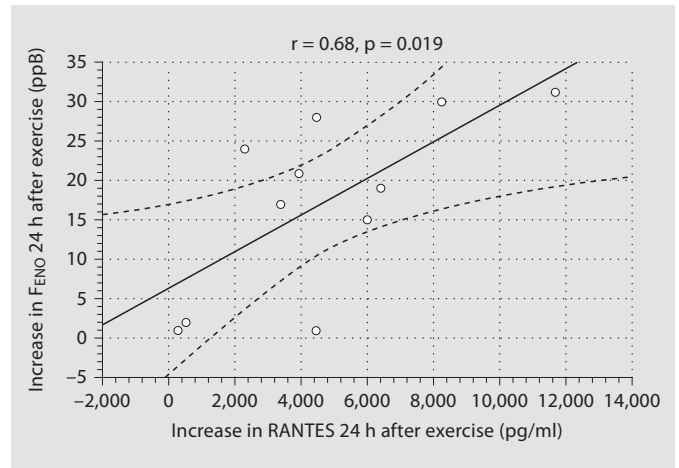
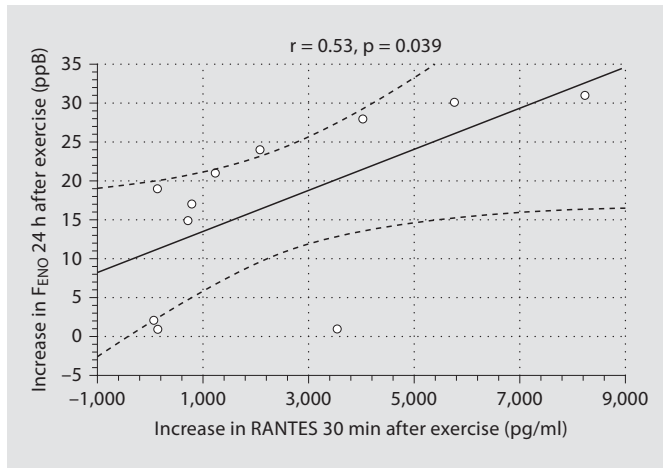


Fig. 4. Correlations between the increase in plasma RANTES 30 min after exercise and increase in F_{ENO} , ECP, or BHR to histamine 24 h after exercise.

Fig. 5. Correlations between the increase in plasma RANTES 24 h after exercise and increase in F_{ENO} , ECP, or BHR to histamine 24 h after exercise.

in the mean plasma concentrations of RANTES or β -TG were found within 30 min after a non-specific bronchial provocation test with histamine (data not shown).

Discussion

Platelet activation has been described in many inflammatory diseases, including asthma. These clinical observations have been confirmed by *in vitro* and *in vivo* studies, which revealed that platelets are an integral and necessary component of the inflammatory response [16]. Yamamoto et al. [17] have demonstrated the raised basal levels of platelet activation markers in asthmatic patients. Yasuba et al. [18] revealed that the release of platelet-specific granular products is increased during symptomatic periods of asthma and after bronchial provocation. These changes in the release of platelet-specific chemokines from granular compartments (including β -TG and PF-4) indicate that intravascular platelet activation occurs.

There are several studies in which changes in the function of platelets during an allergen challenge were assessed. Platelet activation can occur early after the onset of the early asthmatic response to allergen [19]. Evidence of platelet-derived mediators in bronchoalveolar lavage fluid several hours after an allergen challenge has been shown [20]. Kowal et al. [21] demonstrated the relationship between intravascular platelet activation in asthmatic patients and the development of allergic inflammation during a late asthmatic reaction in response to allergen exposure.

Human platelets contain both high- and low-affinity receptors for IgE on their membrane [22]. In patients with allergic asthma, compared with healthy persons, the larger proportion of platelets expresses a high-affinity IgE receptor on their surface [23]. The activation of platelets taken from asthmatics via IgE receptor results in the release of inflammatory mediators, revealing that a non-thrombotic pathway for platelet activation exists [22].

Experimental models of allergic inflammation have revealed a requirement for platelets in pulmonary leukocyte recruitment – this is dependent on the expression of platelet P-selectin [24]. Jawien et al. [25] demonstrated the ability of platelets to bind to eosinophils. Platelets form heterogeneous platelet-leukocyte complexes in the circulation after an allergen challenge and may facilitate eosinophil tethering to endothelium [26]. The activated platelets can influence inflammatory cell recruitment during the late asthmatic reaction. Platelets are recruited to the lungs immediately following allergen exposure in

an experimental animal model. Platelets migrate extravascularly in response to a sensitizing allergen via a mechanism dependent on the interaction between allergen, allergen-specific IgE and the Fc ϵ RI, and this may allow them to participate directly in allergic tissue inflammation [27]. It has been shown that platelets can induce histamine release from mast cells during intravenous administration of an allergen [28]. It has also been demonstrated that allergen exposure in asthmatic patients can elicit platelet migration through tissue to the inflammatory site [29] and their ability to deliver costimulatory signals to antigen presentation cells [30] as well as to communicate with T cells [31].

Exercise is known to have a considerable effect on blood hemostasis [32]. A number of reports have been published on the effect of exercise on platelet activity [10]. During exercise-induced platelet activation, many chemotactic substances are released for neutrophils and monocytes (e.g. serotonin, thromboxane A₂, adenine nucleotides, platelet-derived growth factor). Platelet activation markers (e.g. β -TG and PF-4) are also secreted [10].

Mant et al. [33] have reported that, in normal subjects, short maximal exercise causes neither platelet activation nor altered platelet activity, however it causes a transient increase in the platelet count. In opposition to these results, heavy exercise is reported to have a significant platelet-activating effect, which is evident in both patients with stable angina pectoris and matched healthy controls [34]. After morning exercise, a significant increase in the platelet count was reported. This increase could be caused by the fresh release of platelets from the spleen, bone marrow, and lungs [35].

β -TG is a marker protein that is released from platelet α -granules simultaneously during any activation process [10]. This occurrence can be explained by the endogenous release of ADP and/or epinephrine and their surface receptor sensitivity to adhesion [10]. Though its functional relevance remains unclear, it is very likely that exercise-induced platelet activation may likewise cause neutrophil-platelet aggregate formation [36].

Ahmadizad and El Sayed [37] have revealed that heavy resistance exercise is followed by an increase in PLT (platelet count), PCT (platelet-crit), and MPV (mean platelet volume). This occurred in parallel with an *in vivo* activation of platelet as manifested by an increase in platelet aggregation and a rise in β -TG. These changes could be explained partially by changes in plasma volume and platelet count induced by exercise [38].

Wilkens et al. [39] assessed the effects of a specific PAF acether antagonist on the early asthmatic response to ex-

ercise in asthmatic patients with EIB. The authors revealed a significantly lower increase in PF-4 and β -TG concentrations after an exercise challenge in patients treated with a PAF antagonist when compared with a placebo group. The results of this study confirmed that during EIB in asthmatic patients, platelet activation occurs and PAF is one of the mediators involved in postexercise bronchoconstriction.

RANTES, a member of CCL chemokines, is produced by endothelium cells, fibroblasts, T lymphocytes, eosinophils, and other cells [40]. Previous studies have revealed that an important source of RANTES could be also platelets [41, 42]. RANTES is a powerful chemoattractant of eosinophils, T lymphocytes and basophils [40]. It also activates these immune cells and induces the exocytosis of bronchoconstrictive mediators, such as histamine and cysteinyl leukotrienes from basophils, and eosinophilic cationic protein from eosinophils [40]. Therefore, RANTES might be involved in inflammatory cell recruitment and the induction of bronchoconstrictive mediators release from cells, resulting in airflow limitation.

Our results also confirm the occurrence of platelet activation during postexercise bronchoconstriction in asthmatic patients. This observation could have very important clinical implications, because platelets are a rich source of a wide range of biologically active mediators capable of inducing or augmenting allergic inflammatory responses [3]. The similar pattern of the increase in plasma concentrations of RANTES and β -TG presented in our study indicates that a major source of RANTES could be exactly activated platelets.

Many reports are focused on the importance of the inflammatory process related to eosinophil activation in the pathogenesis of EIB in asthma. Kivity et al. [43] have demonstrated significant increases in sputum eosinophil counts in patients with EIB, but not in those with no EIB or after BPT with metacholine.

The role of chemokines in postexercise bronchoconstriction still remains unclear. Tahan et al. [44] have revealed that, in children with asthma, any significant changes in the chemokine profiles did not occur during postexercise bronchoconstriction. However, the authors point out that these changes could have occurred in the case of a much stronger exercise response than that observed in the patients studied. This assumption could be confirmed by previous studies, which reveal that systemic levels of chemokines may fluctuate after marathon running [45].

Our results indicate that in asthmatic patients, as a consequence of postexercise bronchoconstriction, an increase in RANTES plasma concentrations occurs; this increase could be caused by platelet activation. In the group of asthmatic patients without EIB, as well as in healthy volunteers, 30 min after exercise we observed elevated levels of β -TG. However, β -TG concentrations were not so markedly increased compared with asthmatics with EIB, and did not coexist with the elevated RANTES levels. In the group of asthmatics with EIB, 24 h after exercise, a further increase in RANTES concentration was observed, which could be caused by prolonged stimulation resulting from release of this chemokine. In the group of asthmatics with EIB, the elevated RANTES levels significantly correlated with the increase in airway inflammation markers observed 24 h after exercise.

Our results indicate that platelet activation, and the increase in RANTES release probably related to this activation, could be one of the factors responsible for the intensification of airway inflammation, observed in consequence of postexercise bronchoconstriction in asthmatic patients. More studies are needed to establish the precise role of platelet activation in the pathogenesis of EIB and to develop possible new therapeutic strategies.

References

- 1 Busse WW, Lemanske RF: Asthma. *N Engl J Med* 2001;344:350–362.
- 2 Beasley R, Roche WR, Roberts JA, Holgate ST: Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989;139:806–817.
- 3 Klinger MH, Wilhelm D, Bubel S, Stichering M, Schröder JM, Kühnel W: Immunocytochemical localization of the chemokines RANTES and MIP-1 α within human platelets and their release during storage. *Int Arch Allergy Immunol* 1995;107:541–546.
- 4 Kameyoshi Y, Dorchner A, Mallet AI, Christophers E, Schröder JM: Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J Exp Med* 1992;176:587–592.
- 5 Luster AD: Chemokines: chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436–445.
- 6 Pods R, Ross D, van Hulst S, Rudack C, Maune S: RANTES, eotaxin and eotaxin-2 expression and production in patients with aspirin triad. *Allergy* 2003;58:1165–1170.
- 7 Yoshikawa T, Shoji S, Fujii T, Kanazawa H, Kudoh S, Hirata K, Yoshikawa J: Severity of exercise-induced bronchoconstriction is related to airway eosinophilic inflammation in patients with asthma. *Eur Respir J* 1998;12:879–884.
- 8 Soo-Yeon L, Hyo-Bin K, Ja-Hyung K, Bong-Seong K, Mi-Jan K, Seong-Ok J, Soo-Jong H: Eosinophils play a major role in the severity of exercise-induced bronchoconstriction in children with asthma. *Pediatr Pulmonol* 2006;41:1161–1166.

- 9 Zietkowski Z, Bodzenta-Lukaszyk A, Tomasiak MM, Skiepkowski R, Szmikowski M, Mroczko B: The role of endothelium-derived mediators in exercise-induced bronchoconstriction. *Int Arch Allergy Immunol* 2007; 143:299–310.
- 10 Kestin AS, Ellis PA, Barnard MR, Errichetti A, Rosner BA, Michelson AD: Effect of strenuous exercise on platelet activation state and reactivity. *Circulation* 1993;88:1502–1511.
- 11 Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention: NHLBI/WHO Report 2005, Publ 02-3659 (www.ginasthma.org).
- 12 American Thoracic Society: Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis* 1991;144:1202–1218.
- 13 Ryan G, Dolovich MB, Roberts RS, Frith PA, Juniper EF, Hargreave FE, Newhouse MT: Standardization of inhalation provocation tests: two techniques of aerosol generation and inhalation compared. *Am Rev Respir Dis* 1981;123:195–199.
- 14 Eggleston PA: Methods of exercise challenge. *J Allergy Clin Immunol* 1984;73:666–669.
- 15 American Thoracic Society/American Lung Association Recommendations for online measurement of exhaled nitric oxide in adults and the recommendations for online, offline and nasal expired nitric oxide measurements in children. *Am J Respir Crit Care Med* 1999;160:2104–2117.
- 16 Pitchford SC, Page CP: Platelet activation in asthma: integral to the inflammatory response. *Clin Exp Allergy* 2006;36:399–401.
- 17 Yamamoto H, Nagata M, Tabe K, Kimura I, Kiuchi H, Sakamoto Y, Yamamoto K, Dohi Y: The evidence of platelet activation in bronchial asthma. *J Allergy Clin Immunol* 1993;91:79–87.
- 18 Yasuba H, Chihara J, Kino T, Satake N, Oshima S: Increased releasability of platelet products and reduced heparin-induced platelet factor-4 release from endothelial cells in bronchial asthma. *J Lipid Mediat* 1991;4:5–21.
- 19 Knauer KA, Lichtenstein LM, Adkinson NF Jr, Fish JE: Platelet activation during antigen-induced airway reactions in asthmatic subjects. *N Eng J Med* 1981;304:1404–1406.
- 20 Averill FJ, Hubbard WC, Proud D, Gleich GJ, Liu MC: Platelet activation in the lung after antigen challenge in a model of allergic asthma. *Am Rev Respir Dis* 1992;145:571–576.
- 21 Kowal K, Pampuch A, Kowal-Bielecka O, DuBuske LM, Bodzenta-Lukaszyk A: Platelet activation in allergic asthma patients during allergen challenge with *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2006;36:426–432.
- 22 Joseph M, Capron A, Ameisen JC, Capron M, Vorng H, Pancré V, Kusnier JP, Auriault C: The receptor for IgE on blood platelets. *Eur J Immunol* 1986;16:306–312.
- 23 Joseph M, Gounni AS, Kusnier JP, Vorng H, Sarfati M, Kinet JP, Tonnel AB, Capron A, Capron M: Expression and functions of the high-affinity IgE receptor on human platelets and megakaryocyte precursors. *Eur J Immunol* 1997;27:2212–2218.
- 24 Pitchford SC, Momi S, Giannini S, Casali L, Spina D, Page CP, Gesele P: Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. *Blood* 2005;105:2074–2081.
- 25 Jawien J, Chlopicki S, Gryglewski RW: Interactions between human platelets and eosinophils are mediated by selectin-P. *Pol J Pharmacol* 2002;54:157–160.
- 26 Ulfman LH, Joosten DPH, Van Aalst CW, Lammers JW, van de Graaf EA, Koenderman L, Zwaginga JJ: Platelets promote eosinophil adhesion of patients with asthma to endothelium under flow conditions. *Am J Respir Cell Mol Biol* 2003;28:512–519.
- 27 Pitchford SC, Momi S, Baglioni S, Casali L, Giannini S, Rossi R, Page CP, Gesele P: Allergen induces the migration of platelets to lung tissue in allergic asthma. *Am J Respir Crit Care Med* 2008;177:604–612.
- 28 Yoshida A, Ohba M, Wu X, Sasano T, Nakamura M, Endo Y: Accumulation of platelets in the lung and liver and their degranulation following antigen-challenge in sensitized mice. *Br J Pharmacol* 2002;137:146–152.
- 29 Czapiga M, Gao J-L, Kirk A, Lekstrom-Himes J: Human platelets exhibit chemotaxis using functional N-formyl peptide receptors. *Exp Hematol* 2005;33:73–84.
- 30 Czapiga M, Kirk A, Lekstrom-Himes J: Platelets deliver costimulatory signals to antigen-presenting cells: a potential bridge between injury and immune activation. *Exp Hematol* 2004;32:135–139.
- 31 Danese S, de la Motte C, Rivera Reyes BM, Sans M, Levine AD, Fiocchi C: Cutting edge: T-cell trigger CD40-dependent platelet activation and granular RANTES release: a novel pathway for immune response amplification. *J Immunol* 2004;172:2011–2015.
- 32 El Sayed MS, Sale C, Jones PG, Chester M: Blood hemostasis in exercise and training. *Med Sci Sports Exerc* 2000;32:918–925.
- 33 Mant MJ, Kappagoda CT, Quinlan J: Lack of effect of exercise on platelet activation and platelet reactivity. *J Appl Physiol* 1984;57:1333–1337.
- 34 Wallen NH, Held C, Rehnqvist N, Hjemdahl P: Effects of mental and physical stress on platelet function in patients with stable angina pectoris and healthy controls. *Eur Heart J* 1997;18:807–815.
- 35 El Sayed MS: Effects of exercise on blood coagulation, fibrinolysis and platelet aggregation. *Sports Med* 1996;22:282–298.
- 36 Ott I, Neumann FJ, Gawaz M, Schmitt M, Schömig A: Increased neutrophil-platelet adhesion in patients with unstable angina. *Circulation* 1996;94:1239–1246.
- 37 Ahmadizad S, El Sayed MS: The effects of graded resistance exercise on platelet aggregation and activation. *Med Sci Sports Exerc* 2003;35:1026–1032.
- 38 Ahmadizad S, El Sayed MS, MacLaren DP: Responses of platelet activation and function to a single bout of resistance exercise and recovery. *Clin Hemorheol Microcirc* 2006;35:159–168.
- 39 Wilkens H, Wilkens JH, Uffmann J, Bövers J, Fröhlich JC, Fabel H: Effect of the platelet-activating factor antagonist BN 52063 on exertional asthma. *Pneumologie* 1990;44:347–348.
- 40 Chung KF, Barnes PJ: Cytokines in asthma. *Thorax* 1999;54:825–857.
- 41 Von Hundelshausen P, Petersen F, Brandt E: Platelet-derived chemokines in vascular biology. *Thromb Haemost* 2007;97:704–713.
- 42 Chihara J, Yasuba H, Tsuda A: Elevation of the plasma level of RANTES during asthma attacks. *J Allergy Clin Immunol* 1997;100:52–55.
- 43 Kivity S, Argaman A, Onn A, Shwartz Y, Man A, Greif J, Fireman E: Eosinophil influx into the airways in patients with exercise-induced asthma. *Respir Med* 2000;94:1200–1205.
- 44 Tahan F, Karaaslan C, Aslan A, Kiper N, Kalayci O: The role of chemokines in exercise-induced bronchoconstriction in asthma. *Ann Allergy Asthma Immunol* 2006;96:819–825.
- 45 Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK: Chemokines are elevated in plasma after strenuous exercise in humans. *Eur J Appl Physiol* 2001;84:244–245.