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Changes in Mucosal and Humoral Atopic-Related Markers and Immunoglobulins in Elite Cyclists Participating in the *Vuelta a España*

Abstract

Atopic-related factors, humoral and mucosal immunoglobulins (Ig), and cortisol were measured in 17 professional cyclists competing in the 2003 *Vuelta a España* (a three-week multi-stage race). Venous blood and saliva samples were obtained the morning before the start of the race (T0), on the first rest day after 10 days of racing (T1), and before the start of the last stage after 21 days of racing (T2). Atopic-related factors, IgE, eosinophil cationic protein (ECP), and eosinophils, were significantly altered during the race. Serum IgE (T1: +10%) and ECP (salivary, T1: 113% and serum, T2: 155%) were significantly increased, while eosinophils (T1: -32%, T2: -55%) were significantly lower, than pre-race levels. Salivary sIgA secretion rate was significantly decreased at T2 (-36%). Pearson product-moment correlations re-

vealed a modest correlation between salivary sIgA and salivary ECP (T1: $r = 0.30$; T2: $r = 0.48$; $p < 0.01$). Serum IgM, total IgG, IgG1, IgG2, IgA levels, at T1 and T2, and cortisol at T2, were significantly lower than pre-race levels. In conclusion, the elevation in IgE and ECP suggests an up-regulation of atopic-related factors in professional cyclists participating in the *Vuelta a España*. The correlation between salivary sIgA and salivary ECP indicates a role for sIgA in mediating mucosal inflammation. The alterations in Ig levels may indicate Ig isotype switching. An increasing state of hormonal fatigue may have influenced the observed immune alterations.

Key words

Atopy · mucosal immunity · airway inflammation · cycling

Introduction

Exercise enhances or suppresses the immune system in a specific manner, depending, amongst many other factors, on the mode and duration of exercise [23]. However, the clinical significance of exercise-induced alterations in the immune system is unclear [23]. Although it has been suggested that there is an increased incidence of upper respiratory tract symptoms (URTS) after strenuous, endurance exercise [28] and that exhaustive exercise

might lead to an allergy reaction or atopic disposition [35,36], no specific link has been established [23].

IgE is one of the major mediators of immediate hypersensitivity reactions that underlie atopic conditions such as urticaria, seasonal allergy, asthma, and anaphylaxis [5]. IgE mediates these conditions through inducing the degranulation of mast cells and eosinophils, with the release of stored products, including histamine and eosinophil cationic protein (ECP) [5]. Eosinophils and

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Bibliography

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ECP are believed to be major factors effecting tissue damage to the airway epithelium in allergic asthma, and there is a relationship between eosinophil infiltration/activation and hyperresponsiveness in the airways [31].

Asthma, airway hyperresponsiveness (AHR), anaphylaxis, and systemic histamine release by mast cells are prevalent in athletes [36], while eosinophil activation, with an increase in serum ECP, has been reported after exercise [7,8,24]. Recently, it was hypothesized that tissue damage induced by strenuous exercise may induce an up-regulation of humoral immunity with an increase in IgE synthesis and eosinophil activation [35]. However, this hypothesis is yet to be tested, and therefore, numerous questions remain regarding the relationship between exercise and atopic-related responses [36].

Immunoglobulins (Ig) are the major components of the adaptive humoral and mucosal immune system. Depending on the Ig isotype measured [22], moderate physical activity either stimulates or has no effect on Ig, whereas heavy exercise or periods of intense exercise training are associated with the suppression of Ig levels [15,22]. This relationship has been demonstrated for secretory (s) IgA in the saliva.

slgA is the most prominent Ig isotype in the airways where it serves as a first line of defence by neutralizing pathogens and inhibiting their adhesion to the mucosa [37]. Individuals with selective slgA-deficiency suffer a high incidence of upper respiratory tract infections (URTI). In athletes, however, a direct cause and effect relationship between low slgA and illness has not been shown, prompting the suggestion that it should only be viewed as a risk factor for developing infection [15].

Traditionally, slgA has been regarded as a non-inflammatory antibody, as no inflammatory response is initiated when slgA interacts with pathogens and/or indigenous microflora and dietary antigens [37]. This characteristic of slgA is crucial for preventing the development of chronic inflammatory responses in the mucosa [37]. However, recently it has been shown that slgA is the most potent stimulus for eosinophil activation [26]. Therefore, it has been proposed that slgA may also have a pro-inflammatory function, and may be implicated in the aggravation of allergic reactions through activating eosinophils in the airways [37].

The *Vuelta a España* is an extreme endurance competition that combines both long duration and high intensity bouts of exercise, placing extreme energetic demands on participants. Although many physiological and hormonal aspects of the race have been assessed [11,21], no study has examined alterations in immune response.

Therefore, the primary objective of the study was to determine whether there was an alteration in atopic-related factors such as IgE, eosinophils, and ECP in response to the *Vuelta a España*. Secondly, we were interested in determining whether the tour altered humoral and/or mucosal adaptive immunity, through measuring Ig isotype alterations. Thirdly, due to the possible role that slgA may play in activating eosinophils, our third objective was to examine correlations between systemic and mucosal levels of IgA, IgE, ECP.

Table 1 Nature and incidence of allergy (n = 10/17 cyclists)

Allergy	Incidence
Pollen allergy and asthmatic	4
Asthmatic and exercise induced asthma	3
Allergic rhinitis (nasal congestion and sinus pain)	2
Gastrointestinal symptoms related to a food allergy	1

Materials and Methods

Subjects and race description

The study population was made up of 17 male cyclists from two professional teams. Written consent was obtained from all participants and all procedures were approved by the Ethical Committee of the European University of Madrid.

Subjects' age, height, and weight (mean $\pm$ SD) as at the start of the competition were 28 ± 3 years, 178.2 ± 4.6 cm, and 67.6 ± 5.2 kg. Maximum oxygen consumption ($\dot{V}O_{2\max}$) was assessed using a standard cycle ergometer protocol ($\dot{V}O_{2\max} = 74.4 \pm 4.3$ ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$) [21]. The cardiovascular health of each participant was established by a normal physical examination (including electrocardiogram and echocardiogram) before the start of the tour. No participant had a personal or family history of endocrine disorders. No exogenous substances which could interfere with hormonal function were detected during routine "urine and anti-blood doping" analysis. The incidence of allergy was recorded and it was noted that ten (60%) of the cyclists were asthmatic or allergic in some form (Table 1), four from Team 1 and six from Team 2. The team physicians also monitored the incidence of URTI during the competition and for two weeks after completion of the race.

The *Vuelta a España* starts in late August on the Spanish Mediterranean coast and finishes in mid September. It includes 21 daily stages with only two days of rest. During this period, a total of 2956 km is covered. The mean distance covered per stage for the 2003 race was 141 ± 55 km at altitudes of 0–2200 m above sea level, and the mean daily km covered during the first, second, and third week were 126 ± 63 km, 155 ± 46 km, and 141 ± 59 km, respectively. The average stage speed of the overall winner was 43.3 km $\cdot$ h $^{-1}$. The total time spent during the race by the overall winner was ~ 68 hours. Daily stages started at 12:00 p.m. and finished between 16:30 p.m. and 17:30 p.m. Temperatures usually ranged from 18 to 30°C during the stages.

Research design

The total training of these cyclists in a year ranges from 30 000 to 37 000 km. The cyclists had performed 12.2 ± 2.7 competition days in the previous month. They underwent a tapering week before the start of the race ($3-4$ h $\cdot$ day $^{-1}$ of easy-to-moderate bicycling, the great majority of which was performed at heart rates below 150 beats $\cdot$ min $^{-1}$).

Three venous blood samples (15 ml) and three saliva samples were obtained from each participant during the competition as

follows: at T0 (one day before the competition), T1 (on the first rest day after 10 days of racing), T2 (before the start of the last stage after 21 days of racing). All sampling was performed after an overnight fast and 9 h of sleep (from 12:00 a.m. to 9:00 a.m.) between 09:00 a.m. and 09:30 a.m. and at least 18 hours after the last bout of exercise.

Saliva was collected prior to blood sampling according to a standardised procedure [29]. Subjects were allowed to drink water ad libitum before, with the exception of a 15-minute period prior to each saliva collection. Each subject was asked to swallow in order to empty the mouth before a saliva sample was collected. Unstimulated whole-saliva samples were then collected over a 4-min period into pre-weighed tubes. These samples were then weighed, with saliva volume being measured to the nearest 0.1 ml. The samples were spun at $1500 \times g$ for 15 min at room temperature. The clear sample was then separated by pipette into appropriate tubes and stored at -80°C until analysis.

Blood samples were drawn from an antecubital vein with subjects in a seated position. Routine complete blood counts (CBC) were performed by a clinical haematology laboratory, and provided leukocyte subset counts. For Ig and ECP analysis, blood samples were allowed to clot at room temperature and then centrifuged at $2000 \times g$ for 20 min at 4°C . Separated serum was then aliquoted (0.5 ml) and stored at -80°C until analysis.

Nutrition, hydration, timing of food intake and sleep on the days prior to sampling were held constant, which is normal practice in cycling competitions, in order to maintain circadian rhythms [14,16].

Immunoglobulin

Salivary secretory (S) IgA ($\mu\text{g IgA} \cdot \text{ml}^{-1}$) was determined using an indirect enzyme immunoassay kit (Salimetrics, State College, USA). The concentration of saliva was expressed in two ways: 1) absolute concentration of salivary sIgA ($\mu\text{g sIgA} \cdot \text{ml}^{-1}$); 2) salivary sIgA secretion rate (s-IgA) ($\mu\text{g sIgA} \cdot \text{min}^{-1}$), or the total amount of sIgA appearing on the mucosal surface per time unit. sIgA secretion rate was calculated by multiplying absolute sIgA concentration by saliva flow rate ($\text{ml} \cdot \text{min}^{-1}$). Saliva flow rate was calculated by dividing the total amount of saliva obtained in each sample (ml) by the time taken to produce the sample (4 min) [29]. All samples were analysed in duplicate with the inter- and intra-assay coefficients of variation lower than 3.5% and 3.7%, respectively.

The total amount of serum IgM, IgA, IgG, and IgG subclasses was quantified by incubation with appropriate antisera (anti-human IgM, IgA, IgG and IgG1, IgG2, IgG3, and IgG4 – Behring, Germany). The amount of complex (immunoglobulin-anti immunoglobulin) formed was measured by light scatter, using laser nephelometry (Behring, Germany) and the amount of antibody present, quantitated by comparison with standards of known concentration. All samples were analysed in triplicate with the inter- and intra-assay coefficients of variation lower than 1.9% and 2%, respectively.

The total serum IgE and salivary IgE was determined with the use of the Alastat Microplate Total IgE kit (Diagnostic Products, USA)

according to the manufacturer's instructions and by comparisons with a known range of standard IgE concentrations. All samples were analysed in triplicate with the inter- and intra-assay coefficients of variation lower than 2% and 2.5%, respectively for serum IgE, and lower than 4.3% and 5.2% for salivary IgE.

Serum and salivary eosinophil cationic protein (ECP)

Serum and salivary ECP concentrations were determined by a radioimmunoassay technique (ECP-RIA; Pharmacia; Stockholm, Sweden) as described by Petersen [30]. All samples were analysed in triplicate with the inter- and intra-assay coefficients of variation lower than 3% and 3.1%, respectively for serum ECP, and lower than 4.8% and 5.5% for salivary ECP.

Cortisol

Serum cortisol concentrations were measured by competitive chemiluminescence immunoassay (Bayer ADVIA Centaur® immunoassay system; Bayer HealthCare LLC Diagnostic Division, Tarrytown, NY, USA). The coefficients of inter-assay and intra-assay variability averaged 3.23% and 2.97%, respectively.

Statistical analysis

Due to the possibility that an allergic/atopic disposition may alter the way that the immune system responds to exercise it was decided to group athletes into an atopic group and a normal group to determine any significant differences in response to the cycling competition. Since there was no significant interaction or group effects, all subjects were combined into one group. A repeated measures analysis of variance (ANOVA, 1×3) was used (SAS, Cary NC, USA) to determine whether there were any significant differences ($p < 0.05$) across time for the serum and saliva variables. When appropriate, Bonferroni post hoc tests were performed. Pearson product-moment correlations were used to test the relationship between serum and salivary variables, specifically serum IgA and salivary sIgA, serum ECP and salivary ECP, serum IgE and salivary IgE, as well as between salivary ECP and salivary sIgA.

Results

The nature and incidence of allergy is shown in Table 1. There were no reports of URTI during or for two weeks after the race. Plasma volume was not significantly different and body mass was maintained near pre-race levels over all three sampling time points (individual changes $< 10\%$ and $< 5\%$, respectively). Subjects experienced a moderate neutrophilia (T1: $+17\%$, T2: $+19\%$ increase), however lymphocytes (T1: -16% , T2: -15%), monocytes (T1: -50% , T2: -33%), and eosinophils (T1: -32% , T2: -55%) all decreased from pre-race levels (Table 2). These responses were all significant ($p < 0.01$).

Immunoglobulin

The pre- and post-race levels of serum IgM, IgA, total IgG, IgG2, IgG3, IgG4 were at the low end of the laboratory established normal reference ranges (Figs. 1–4) at all three sample times. IgG1 level was 2–14% lower, while IgE was 40 to 50% higher, than the normal range at all three sample times. Significant alterations in serum Ig level were found during the race compared to pre-race levels.

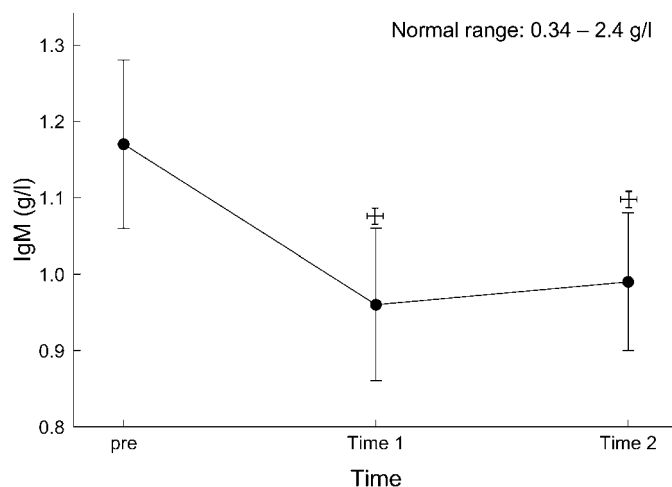


Fig. 1 Immunoglobulin M level (mean $\pm$ SE). Note: *, $p \leq 0.0001$, significant change from pre-race.

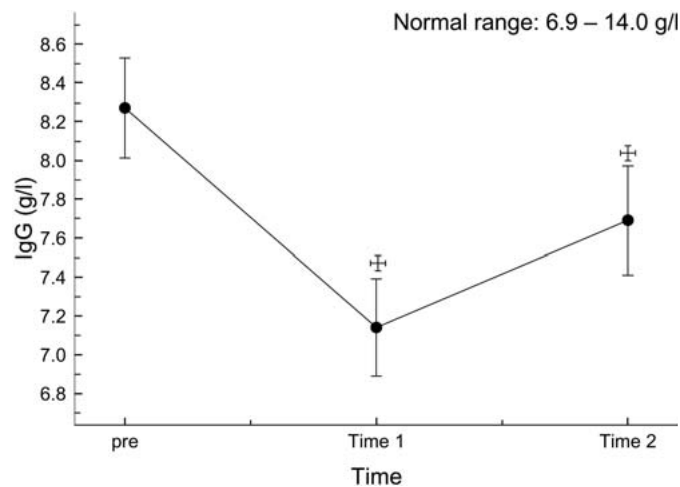


Fig. 2 Immunoglobulin G level (mean $\pm$ SE). Note: *, $p \leq 0.01$, significant change from pre-race.

Table 2 Leukocyte subset counts (n = 17) (mean $\pm$ SE)

Variable	T0 (pre-race)	T1 (1st rest day)	T2 (last stage)
Total leukocytes ($10^9/l$)	6.23 $\pm$ 0.74	6.65 $\pm$ 0.47*	7.63 $\pm$ 0.49*
Neutrophils (%)	46.12 $\pm$ 2.81	55.50 $\pm$ 2.41*	57.03 $\pm$ 2.07*
Lymphocytes (%)	41.51 $\pm$ 2.91	35.03 $\pm$ 2.82*	35.29 $\pm$ 1.99*
Monocytes (%)	8.61 $\pm$ 0.69	4.34 $\pm$ 0.59*	5.75 $\pm$ 0.28*
Eosinophils (%)	3.17 $\pm$ 0.52	2.17 $\pm$ 0.78*	1.42 $\pm$ 0.32*

* $p < 0.05$, change from pre-race

Immunoglobulin A

Serum IgA concentration was significantly lower at T1 and T2 compared to the pre-race level. Serum IgA decreased significantly from 1.84 ± 0.18 g/l pre-race to 1.65 ± 0.14 g/l at T1 (10% decrease, $p = 0.0002$) and 1.67 ± 0.14 g/l at T2 (9% decrease, $p = 0.01$) (Table 3).

The saliva flow rate was not significantly different at each of the sampling time points. Salivary sIgA concentration decreased significantly from 662 ± 91 $\mu\text{g} \cdot \text{ml}^{-1}$ pre-race to 469 ± 61 $\mu\text{g} \cdot \text{ml}^{-1}$, at T2 (30% decrease, $p = 0.02$). The salivary sIgA secretion rate decreased significantly from 715 ± 120 $\mu\text{g} \cdot \text{min}^{-1}$ pre-race to 455 ± 69 $\mu\text{g} \cdot \text{min}^{-1}$, at T2 (36% decrease, $p = 0.008$) (Table 3).

Immunoglobulin M

Serum IgM concentration was significantly lower at T1 and T2 compared to the pre-race level. IgM decreased significantly from 1.17 ± 0.11 g/l pre-race to 0.96 ± 0.10 g/l at T1 (18% decrease, $p < 0.0001$), and 0.99 ± 0.09 g/l at T2 (16% decrease, $p = 0.0001$) (Fig. 1).

Immunoglobulin G

Compared to pre-race values, serum IgG was significantly lower during the race. IgG decreased significantly from 8.27 ± 0.26 g/l

pre-race to 7.14 ± 0.25 g/l at T1 (14% decrease, $p < 0.0001$) and 7.69 ± 0.28 at T2 (7% decrease, $p = 0.002$) (Fig. 2).

Immunoglobulin G1, G2, G3, and G4

The levels of IgG1 and IgG2 responded similarly to total IgG during the cycle race. Fig. 3 shows that the IgG1 level decreased significantly from 4.82 ± 0.13 g/l pre-race to 4.21 ± 0.15 g/l at T1 (13% decrease, $p < 0.0001$) and 4.54 ± 0.18 at T2 g/l (6% decrease, $p = 0.003$). IgG2 decreased significantly from 2.55 ± 0.18 g/l pre-race to 2.11 ± 0.15 g/l at T1 (18% decrease, $p < 0.0001$) and 2.24 ± 0.16 g/l at T2 (13% decrease, $p = 0.003$). Fig. 3 shows that IgG3 and IgG4 were not altered during the cycle race.

Immunoglobulin E

Fig. 4 shows that the serum IgE level was increased significantly from 145 ± 38 KU/l pre-race to 159 ± 38 KU/l at T1 (10% increase, $p = 0.009$). Serum IgE remained elevated at T2, 159 ± 37 KU/l (10% increase) although this value was not significant ($p = 0.3$).

Salivary IgE increased from 0.97 ± 0.5 KU/l pre-race to 2.28 ± 1.55 KU/l at T1 (135% increase) and 2.67 ± 1.41 KU/l at T2 (175%), however the increases were not significant ($p = 0.2$). This was most likely due to the large individual variation found in the salivary IgE levels (Fig. 4).

Eosinophil cationic protein (ECP)

The level of serum ECP increased from 4.57 ± 0.47 $\mu\text{g/l}$ pre-race to 8.39 ± 2.22 $\mu\text{g/l}$ at T1 (84% increase) however this value was not significant. Serum ECP level continued to rise and by T2 had increased to 11.67 ± 2.39 $\mu\text{g/l}$, a 155% increase which was significant ($p = 0.01$) (Fig. 5).

Salivary ECP increased significantly from 7.28 ± 1.50 $\mu\text{g/l}$ pre-race to 15.52 ± 3.90 $\mu\text{g/l}$ at T1 (113% increase, $p = 0.03$) but returned to pre-race levels by T2 (6.39 ± 2.11 $\mu\text{g/l}$). This was a significant decrease from T1 (59% decrease, $p = 0.02$) (Fig. 5).

Cortisol

Serum cortisol decreased significantly from 25.03 ± 1.29 $\mu\text{g/dl}$ pre-race to 20.16 ± 1.96 $\mu\text{g/dl}$ at T2 (20% decrease, $p = 0.04$). Cor-

Table 3 Salivary and serum IgA (n = 17) (mean ± SE)

Variable	T0	T1	T2	Normal range
Saliva flow rate (ml·min ⁻¹)	1.08 ± 0.11	1.08 ± 0.12	0.97 ± 0.11	
SlgA concentration (μg·ml ⁻¹)	662 ± 91	760 ± 140	469 ± 61*	
SlgA secretion rate (μg·min ⁻¹)	715 ± 120	820 ± 165	455 ± 69**	
Serum IgA (g/l)	1.84 ± 0.18	1.65 ± 0.14***	1.67 ± 0.14**	0.7–4.0 g/l

* p < 0.05, ** p < 0.01, *** p < 0.001, change from pre-race

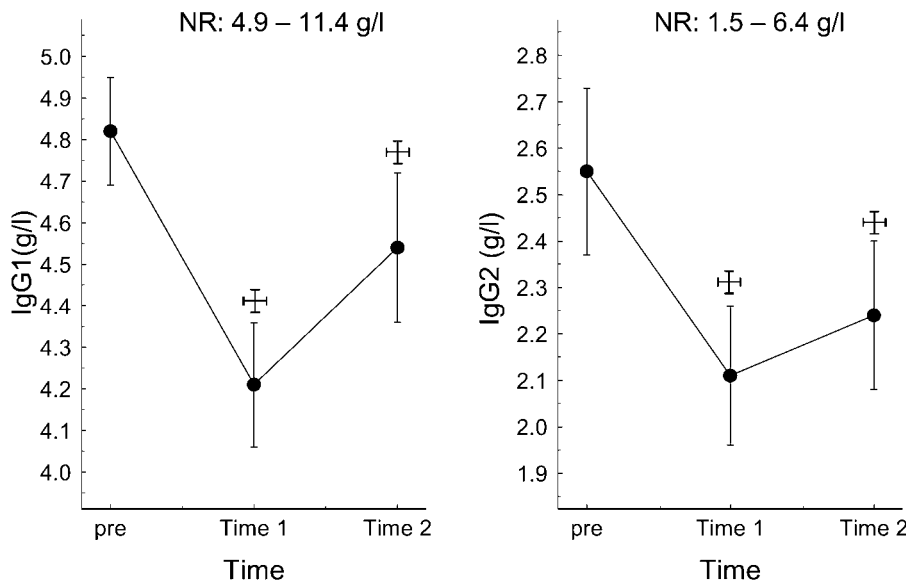


Fig. 3 Immunoglobulin G1, 2, 3, and 4 levels (mean ± SE). Note: *, p ≤ 0.01, significant change from pre-race (NR = normal range).

tisol was also decreased (10%) at T1 ($22.72 \pm 1.31 \mu\text{g/dl}$), however this was not significant (Fig. 6).

Serum and mucosal correlation

Pearson product-moment correlations revealed modest, significant correlations ($p < 0.01$) at T1 ($r = 0.30$) and T2 ($r = 0.48$) between salivary ECP and salivary sIgA concentration, as well as between salivary ECP and salivary sIgA secretion rate (T1: $r = 0.35$, T2: $r = 0.36$). Significant correlations ($p < 0.01$) were also found at T1, but not T2, between serum IgA and salivary sIgA concentration ($r = 0.31$), serum IgA and salivary sIgA secretion rate ($r = 0.31$), and serum ECP and salivary ECP ($r = 0.37$). There was no significant correlation between salivary and serum IgE.

Discussion

This study demonstrated significant alterations in atopic-related factors and mucosal and humoral adaptive immune responses during the *Vuelta a España*. There was a significant increase in IgE (serum) and ECP (serum and saliva), while blood eosinophils and serum IgM, IgG, IgG1, IgG2, IgA, and salivary sIgA were significantly decreased. Interestingly, there was a modest correlation between salivary ECP and salivary sIgA during the race. In addition, cortisol level was significantly decreased by the end of the race, suggesting that the immune alterations occurred during a state of “hormonal fatigue”.

The elevation in IgE and ECP represents an increase in atopic-related factors during the race. This result supports previous findings of a high prevalence of asthma and AHR in the athletic population [19], increased airway inflammatory cells in endurance athletes [2], as well as allergic/atopic disorders in the overtraining syndrome (OTS) [12]. Interestingly, it has been suggested that frequent episodes of ECP peaks may create a higher risk for developing exercise-induced and allergic asthma [7]. However, the effect of repeated episodes of ECP elevation on AHR in healthy subjects has not been established [7]. The above findings suggest that athletic training or strenuous endurance exercise may promote an atopic disposition [36]. However, further research is required to determine the clinical significance of the exercise-induced increase in atopic-related factors.

The link between the hypothalamic-pituitary-adrenal axis and immune function has been well documented [4, 9] and endocrine factors have been shown to influence the immune response to exercise [10, 38]. However, the mechanism underlying the suppression of Ig and antibody in elite athletes undergoing prolonged period of intense exercise is speculative. It has been suggested that the neuroendocrine system plays an important role in Ig and antibody response to exercise [22]. Excessive training has been shown to dampen the sympathetic nervous system (SNS), specifically depleting norepinephrine (NE) production, and response to exercise [20]. NE plays a crucial role in antibody synthesis [33] and therefore, depletion may result in suppressed antibody lev-

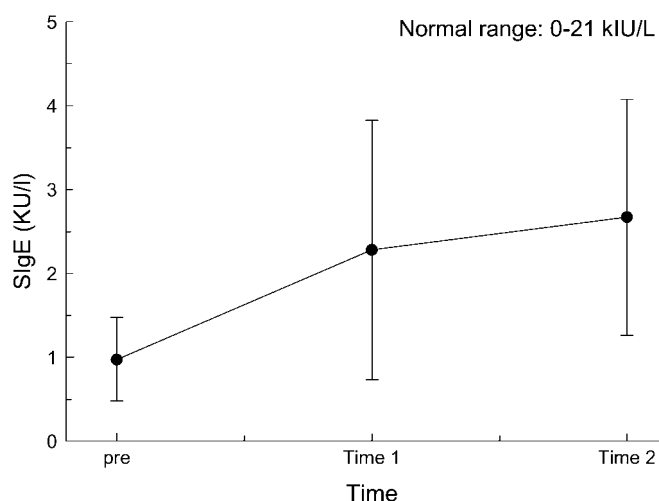


Fig. 4 Serum and salivary immunoglobulin E levels (mean $\pm$ SE). Note: $\ast$; $p < 0.01$, significant change from pre-race.

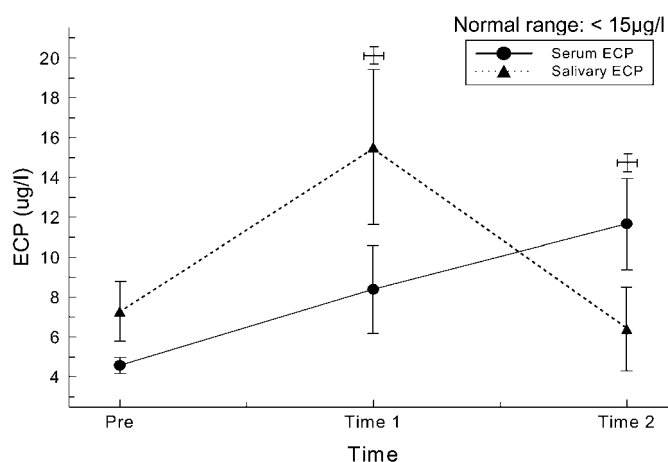


Fig. 5 Serum and salivary ECP levels (mean $\pm$ SE). Note: $\ast$; $p < 0.05$, significant change from pre-race.

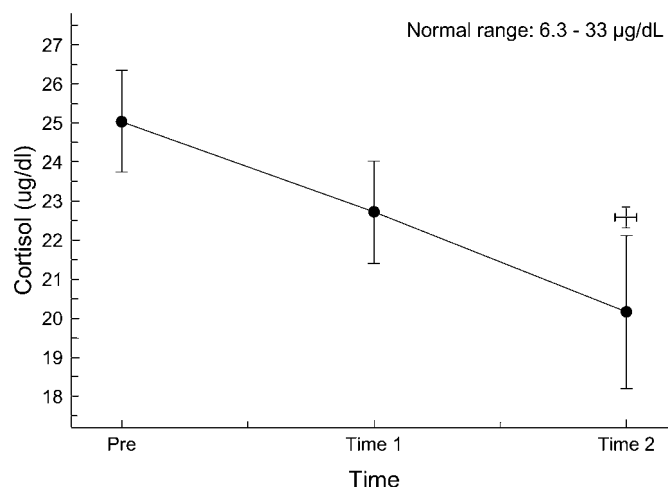


Fig. 6 Cortisol levels (mean $\pm$ SE). Note: $\ast$; $p < 0.05$, significant change from pre-race.

els and an impaired ability to mount an antibody response [22]. The *Vuelta a España* has been shown to induce a state of "hormonal fatigue", dampening the hypothalamic-pituitary-adrenal ax-

is, specifically cortisol levels [11,21]. This state was confirmed once again in the present study. Therefore, it is possible that the SNS and associated release of NE was also dampened, which may explain our suppressed Ig results. In addition, it may be that the absence of adequate levels of cortisol could in part be responsible for the increased level of IgE. Glucocorticoids/corticosteroids are used to treat allergic patients and have been shown to lower serum IgE levels [17,18,27]. It is possible that normal cortisol levels are required to suppress IgE production. However, when cortisol levels are reduced, the status quo is altered and there is an increase in serum IgE.

Exercise-induced Ig isotype switching may be an additional explanation for the elevation in IgE and suppression of IgM, IgA and IgG, IgG1 and IgG2 found in the present study. Although not measured, the T_H2 cytokines, IL-4, IL-6, and IL-13 are responsible for Ig isotype switching of IgM, IgG, or IgA to IgE in atopic individuals [32]. Further research is required to elucidate the relationship between exercise, cytokines, and Ig isotype switching.

Similar to serum Ig, the mechanism responsible for decreases in salivary sIgA, observed after intense exercise, and during periods of intensive training, is speculative [15]. It has been proposed that an alteration or damage to the oral mucosal surfaces due to high ventilatory flow during exercise results in suppressed sIgA secretion. This is possibly due to decreased transport of sIgA across the mucosal epithelium [22].

We would like to propose an additional mechanism for the decreased level of salivary sIgA. Research has demonstrated that sIgA is the most potent activator of eosinophils in the mucosa [3,26]. Although previously regarded as a non-inflammatory antibody, it has recently been proposed to be the principle Ig mediating inflammatory diseases associated with mucosal eosinophils, such as atopic diseases and parasitic infection [25]. There is a positive correlation between eosinophil infiltration into the mucosa and enhanced sIgA production [1,13]. With this relationship in mind we propose that, after strenuous endurance exercise, the binding of sIgA to mucosal eosinophils results in the measurement of a suppressed level of salivary sIgA. Our results support such a mechanism, as not only did we show a modest correlation between decreased salivary sIgA and ECP (saliva and serum), a marker of eosinophil degranulation, but also a decrease in serum eosinophils. It has been suggested that the blood eosinopenia [34], observed immediately after endurance exercise, represents an enhanced migration of serum eosinophils to airway tissues [7]. These "migrated" eosinophils may have been the source of ECP, once they had been activated by sIgA. The finding suggests that sIgA plays an important role in regulating mucosal inflammation. The protective or pathological significance of this after strenuous endurance exercise requires further study.

Conclusion

In summary, the *Vuelta a España* up-regulated atopic-related factors, suggesting the need for additional research into the relationship between exercise and the development of an atopic disposition. In addition the race altered the humoral adaptive immune system, which may have been due to alterations in the

neuroendocrine system, or Ig isotype switching regulated by cytokines. To the best of the authors' knowledge this is the first study to demonstrate a relationship between salivary ECP and salivary sIgA in response to exercise. It is possible that the decrease in salivary sIgA after strenuous endurance exercise may be due to its role in the activation of mucosal eosinophils. Further research is required to elucidate the relationship between exercise, sIgA, and eosinophil activation. This may help explain the recent suggestion that airway inflammation processes may contribute to the increased incidence of URTS in elite distance runners [6]. Finally, these immune alterations occurred during a state of "hormonal" fatigue. This suggests that the *Vuelta a España* represents a unique model for understanding the regulation of immune responses by the hypothalamic-pituitary-adrenal axis.

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References

- Abu-Ghazaleh RI, Fujisawa T, Mestecky J, Kyle RA, Gleich GJ. IgA-induced eosinophil degranulation. *J Immunol* 1989; 142: 2393–2400
- Bonsignore MR, Morici G, Vignola AM, Riccobono L, Bonanno A, Profita M, Abate P, Scichilone N, Amato G, Bellia V, Bonsignore G. Increased airway inflammatory cells in endurance athletes: what do they mean? *Clin Exp Allergy* 2003; 33: 14–21
- Capron M, Desreumaux P. Immunobiology of eosinophils in allergy and inflammation. *Res Immunol* 1997; 148: 29–33
- Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *New Engl J Med* 1995; 332: 1351–1362
- Corry DB, Kheradmand F. Induction and regulation of the IgE response. *Nature* 1999; 402: B18–B23
- Cox AJ, Gleeson M, Pyne DB, Saunders PU, Clancy RL, Fricker PA. Valtrex™ therapy for Epstein-Barr virus reactivation and upper respiratory symptoms in elite runners. *Med Sci Sports Exerc* 2004; 36: 1104–1110
- Domej W, Schwaberg G, Tilz GP, Foldes-Papp Z, Demel U, Lang J, von Duvillard P. Prolonged endurance challenge at moderate altitude. Effect on serum eosinophil cationic protein, eosinophil dynamics, and lung function. *Chest* 2002; 121: 1111–1116
- Dufaux B, Heine O, Prinz U, Soll C, Rost R. Effect of short maximal physical exercise on the eosinophil cationic protein. *Int J Sports Med* 1993; 14: 468–470
- Elenkov IJ, Chrousos GP. Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *TEM* 1999; 10: 359–367
- Eskola J, Ruuskanen O, Soppi E, Viljanen MK, Jarvinen M, Toivonen H, Kouvalainen K. Effect of sport stress on lymphocyte transformation and antibody formation. *Clin Exp Immunol* 1978; 32: 339–345
- Fernandez-Garcia B, Lucia A, Hoyos J, Chicharro JL, Rodriguez-Alonso M, Bandres F, Terrados N. The response of sexual and stress hormones of male pro-cyclists during continuous intense competition. *Int J Sports Med* 2002; 23: 555–560
- Foster C. Monitoring training in athletes with reference to overtraining syndrome. *Med Sci Sports Exerc* 1998; 30: 1164–1168
- Fouda EE. Immunoglobulin A (IgA) in allergic airway disease. *J Allergy Clin Immunol* 2004; 113 (Suppl 1): S328
- Garcia-Roves PM, Terrados N, Fernandez SF, Patterson AM. Macronutrients intake of top level cyclists during continuous competition-change in the feeding pattern. *Int J Sports Med* 1998; 19: 61–67
- Gleeson M, Pyne DB, Callister R. Exercise effects on mucosal immunity and risk of upper respiratory illness. *Int Sport Med J* 2003; 4: 1–13
- Gulledge TP, Hackney AC. Reproducibility of low resting testosterone concentrations in endurance trained men. *Eur J Appl Physiol* 1996; 73: 582–583
- Kampen GT, Poulsen LK, Nielsen HJ, Schulze S, Petersen IJ. IgE levels in surgery: Effect of ranitidine and prednisolone. *Allergy* 1999; 54: 171–176
- Klebl FH, Weber G, Kalden JR, Nusslein HG. In vitro and in vivo effect of glucocorticoids on IgE and IgG subclass secretion. *Clin Exp Allergy* 1994; 24: 1022–1029
- Langdeau J, Boulet L. Prevalence and mechanisms of development of asthma and airway hyperresponsiveness in athletes. *Sports Med* 2001; 31: 601–616
- Lehmann MP, Baumgartl P, Wiesenack C, Seidel A, Baumann J, Fischer S, Spori U, Genmdisch G, Kaminski R, Keul J. Training-overtraining: Influence of a defined increase in training volume vs. training intensity on performance, catecholamines and some metabolic parameters in experienced middle- and long-distance runners. *Eur J Appl Physiol* 1992; 64: 169–170
- Lucia A, Diaz B, Hoyos J, Fernandez C, Villa G, Bandres F, Chicharro JL. Hormone levels of world class cyclists during the Tour of Spain stage race. *Br J Sports Med* 2001; 35: 424–430
- Mackinnon LT. Immunoglobulin, antibody, and exercise. *Exerc Immunol Rev* 1996; 2: 1–35
- Malm C. Exercise immunology: The current state of man and mouse. *Sports Med* 2004; 34: 555–566
- McKune AJ, Smith LL, Semple SJ, Wade AA. Nonallergic activation of eosinophils after strenuous endurance exercise. *SASMA* 2004; 16: 12–16
- Motegi Y, Kita H. Interaction with secretory component stimulates effector functions of human eosinophils but not of neutrophils. *J Immunol* 1998; 161: 4340–4346
- Motegi Y, Kita H, Kato M, Morikawa A. Role of secretory IgA, secretory component, and eosinophils in mucosal inflammation. *Int Arch Allergy Immunol* 2000; 122 (Suppl 1): 25–27
- Naclerio RM, Adkinson NF, Creticos PS, Baroody FM, Hamilton RG. Intranasal steroids inhibit seasonal increases in ragweed-specific immunoglobulin E antibodies. *J Allergy Clin Immunol* 1993; 92: 717–721
- Nieman D, Johanssen LM, Lee JW. Infectious episodes in runners before and after the Los Angeles Marathon. *J Sportsmed Phys Fitness* 1990; 30: 316–328
- Novas AMP, Rowbottom DG, Jenkins DG. Tennis, incidence of URTI and salivary IgA. *Int J Sports Med* 2003; 24: 223–229
- Peterson CG, Enander I, Nystrand J, Anderson AS, Nilsson L, Venge P. Radioimmunoassay of human eosinophil cationic protein (ECP) by an improved method. Establishment of normal levels in serum and turnover in vivo. *Clin Exp Allergy* 1991; 21: 561–567
- Rankin AM, Conroy DM, Williams TJ. Eotaxin and eosinophil recruitment: implications for human disease. *Mol Med Today* 2000; 6: 20–27
- Roitt I, Brostoff J, Male D. *Immunology*. Edinburgh: Mosby, 2001.
- Sanders VM, Munson AE. Norepinephrine and the antibody response. *Pharmacol Rev* 1985; 37: 229–248
- Shepherd RJ. *Physical Activity, Training and the Immune Response*. Carmel (IN): Cooper Publishing Group, 1997
- Smith LL. Overtraining, excessive exercise, and altered immunity: is this a T helper-1 versus T helper-2 lymphocyte response? *Sports Med* 2003; 33: 347–364
- Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exerc Immunol Rev* 2002; 8: 6–48
- van Egmond M, Damen CA, van Spriell AB, Vidarsson G, van Garderen E, van de Winkel JGJ. IgA and the IgA Fc receptor. *Trends Immunol* 2001; 22: 205–211
- Yu DTY, Clements PJ, Pearson CM. Effects of corticosteroids on exercise-induced lymphocytosis. *Clin Exp Immunol* 1977; 28: 326–331