Respiratory tract symptoms in multi-day trail runners – a focus on allergy

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DECLARATION

I, Anton de Waard, declare that the work on which this project is based is original and my own (except where acknowledgements indicate to the contrary) and that neither the whole work or part thereof has been, is being, or is submitted for another degree at this or any other university.

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ABSTRACT

Introduction: Respiratory tract symptoms (RTS), common in athletes during heavy training and after events, result in impaired readiness for events and race times. Since the 1980's exercise immunologists have investigated the aetiological factors surrounding the development of exercise induced RTS in order to develop effective preventative strategies. A number of theories have been put forward and explored, such as increased susceptibility to infection, 'run-away' inflammatory response and reactivation of prior viral infection. It has been suggested that the mechanisms producing exercise induced inflammation could potentiate allergic responses in sensitized individuals and recently allergic response has been proposed as a potential contributor to exercise induced RTS. Certainly allergic reactions can produce a range of respiratory symptoms; however the relationship between allergic sensitization, allergic reaction and the incidence of post-exercise RTS has not been well defined.

Objectives: The primary objective of this study was to document the incidence of RTS for two weeks before and two weeks after a three-day trail run and relate these to the general systemic and salivary immunological profile as well as atopic status of the participants. The secondary objective was to validate the use of the Phadiatop® assay as a predictor of allergy-associated post-race RTS in trail runners.

Study Design and Methods: The study formed part of a larger, descriptive field study examining the physiological responses of trail runners during the Three Cranes Challenge, a multi-day 95 km event divided into three stages, in Karkloof, KwaZulu-Natal. Outcome measures examined included self- reported RTS over a 31 day period (pre, during and post race), as well as pre-race Phadiatop® status, salivary IgA (sIgA) concentrations and changes in concentrations of serum IgE (sIgE), cortisol, high sensitivity C-Reactive Protein (hs-CRP) and differential leukocyte counts. The haematological and salivary parameters were obtained at 8 time points before, during and after the event.

A convenience sample of 22 individuals was used and two separate analyses were conducted on the data. The inclusion criteria of the first analysis were met by 14 participants. In this analysis, the incidence of RTS was related to each participant's general immunological profile. Sixteen of the subjects met the inclusion criteria for the second analysis, in which their Phadiatop® status was related to their sIgE and blood eosinophil and basophil concentrations in order to establish the validity of the Phadiatop® assay in predicting the development of allergy–associated post-exercise RTS in trail runners.

Results: In the first analysis, 78.6 % (n=11) of subjects met the criteria for positive diagnosis of upper respiratory symptoms (URS) during the two week post-race period. In four subjects (36.4 %), URS appeared to be of inflammatory origin, but these were not linked to systemic markers of an allergic response. Of the URS positive subjects, six (54.5 %) presented with markers of infection, three (27.3 %) with markers of a *de novo* infection and three (27.3%) with a profile suggestive of reactivation of previous infection. Of those presenting with markers of infection 66.7 % (n=4) had concomitantly elevated levels of sIgE suggestive of allergic response. There was, however, no evidence of isolated allergic reaction independent of other causes amongst the symptomatic subjects.

In the second analysis, 75% (n=12) of runners presented with post-race RTS and seven of these were Phadiatop® positive. In four of the Phadiatop® positive RTS subjects, symptoms appeared to be of allergic origin. Although total IgE concentrations were significantly higher ($p< 0.01$) in Phadiatop® positive group, there was no significant difference between the eosinophil and basophil concentrations or post-race RTS of the positive and negative groups ($p>0.05$). Of the four subjects who did not develop RTS, three were Phadiatop® positive.

Conclusion: Respiratory tract symptoms in trail runners have a multi-factorial aetiology. A link between concurrent markers of an allergic response and infection is common in symptomatic trail runners. The Phadiatop® assay does not accurately predict the incidence of allergic post-exercise RTS in trail runners.

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CHAPTER 1 – INTRODUCTION

1.1 Background to the problem

It has been noted since the early 1980's that intensive exercise training and exhaustive endurance events result in an increased incidence of Upper Respiratory Tract Infections (URTI) in athletes (Peters and Bateman, 1983). These symptoms interrupt training schedules and impair performance or readiness for competitive events. For many years exercise immunologists have sought methods of identifying the cause of these symptoms (Walsh *et al.,* 2011) and numerous theories have been examined in an attempt to explain them.

As the majority of cases of post-exercise URTI symptoms are now known not be infectious in origin and may include conditions of the lower respiratory tract, the term, respiratory tract symptoms (RTS), is preferred current terminology (Schwellnus *et al.,* 2010). Studies have shown that only approximately one third of cases of post-exercise RTS are caused by infection, another third caused by a non-infectious (inflammatory) medical causes and the final third unknown etiology (Reid *et al.*, 2004; Spence *et al.,* 2007; Cox *et al.,* 2008). Other theories proposed to explain these non-infectious causes include systemic or run-away inflammatory response, reactivation of prior viral infection, development of hypersensitive airways and allergic response.

Athletes experience higher rates of allergic diseases than the general population (Thomas *et al.,* 2010) and the cited incidence of allergy among Olympic athletes is steadily increasing (Weiler *et al.,* 1998; Katelaris *et al.,* 2003; Bonini *et al.,* 2003). A potential link between exercise and an up-regulation of the T_H2 (allergic) side of the immune system was suggested by Smith as early as 2003, while examining the cytokine response of the body to strenuous exercise and tissue trauma. Allergies are typically associated with raised circulating concentrations of IL-4, IL-5, IL-10, and IL-13 and IgE and low concentrations of INFγ (Meischer and Vogel 2002; Neaville *et al.,* 2003). This T_H2 dominant shift has also been associated with a higher incidence of allergies in athletes (Bonini and Craig, 2008). Additionally, it has been demonstrated that atopy is a

major risk factor for hyper-reactive airways (HRA) and exercise induced asthma in athletes, and it has even been suggested that atopy is more common among athletes (Helenius *et al.,* 1998; Langdeau *et al.,* 2000). It has also been proposed that repeated exposure to allergens or damaging pollutants increases the potential for allergic responses in athletes who are predisposed (Bonini *et al.,* 2006).

Numerous studies (Katelaris *et al.,* 2003; Bonini *et al.,* 2006; Bonini and Craig, 2008; Carlsen *et al.,* 2008; Thomas *et al.,* 2008) have highlighted the prevalence of allergic disease amongst the athletic population and recent reviews (Peters, 2004; Schwellnus *et al.,* 2010) have included allergy as a possible contributor to post-exercise upper or lower respiratory tract symptoms. Furthermore, as allergic reactions can produce a range of upper and lower respiratory symptoms that closely mimic URTI (Schwellnus *et al.,* 2010) and as exercise may up-regulate an allergic response in those already sensitized, allergic response should definitely be considered as an underlying factor in the development of post-exercise RTS. This is further reinforced by recent findings of Schwellnus *et al.* (2011) who related the high incidence of pre-race RTS symptoms in triathletes to allergy.

Thus, although there is still much debate as to the cause of a large percentage of post exercise RTS, and while the link between RTS and atopy has been proposed by a number of authors, more research is needed to support this. A clear understanding of the underlying factors or a definitive test to identify those at risk may allow for the development of effective strategies to prevent post-exercise RTS.

1.2 Aims and objectives of the study

The primary objective of this field trial was to document the incidence of self- reported respiratory tract symptoms over a 31 day period (from 2 weeks before the race until 2 weeks after the race) in 22 athletes competing in the Three Cranes Challenge 2011, a three day 95 km trail run, in Karkloof, KwaZulu-Natal, and relate these to the general systemic and salivary immunological profile as well as atopic status of the participants. A secondary objective was to

examine the validity of the Phadiatop® test as a predictor of allergy-associated RTS in these runners.

1.3 Hypotheses

1) Due to the nature of the race (over multiple days and in a conservancy area including forests and grassland) there will be an increase in self-reported RTS in athletes during the two weeks following the event.

2) The three-day trail run will result in cumulative effects on systemic markers of immune system activation.

3) The incidence of RTS would not be related to one individual systemic or salivary marker of immune function.

4) Atopic runners (diagnosed using the Phadiatop® assay), will present with raised markers of allergic activation.

5) The Phadiatop® assay accurately predicts predisposition to RTS of allergic origin.

1.4 Scope of the work

A convenience sample of 22 (15 female, 7 male) subjects between the ages of 25 and 50 years, made up of entrants into the Three Cranes Challenge 2011, a 95 km trail run divided into three daily stages, in Karkloof, KwaZulu-Natal, volunteered to participate in this study. Only 16 subjects completed the in-race and post event testing, and a further two were excluded due to reliance on anti-histamine medication.

The athletes were required to document the incidence of self- reported RTS over a 31 day period (beginning 2 weeks before the race and ending 2 weeks after). Blood samples were collected at a total of eight time-points, before and after each day's stage, and at 24 hours and 72 hours post race. Saliva samples were collected between 60-90 minutes before the start of the 1st stage of the race within 60 minutes following completion of the last stage of the race, and at 24 and 72 hours post race. Outcome measures examined included incidence of RTS, pre-race Phadiatop® status, changes in concentrations of sIgE, cortisol, high sensitivity C - reactive protein (hs-CRP), differential leukocyte counts and sIgA.

Two analyses of the data were conducted. In the first, the incidence of RTS was related to the detailed immunological profile of the athletes, in 14 case studies. In the second, the validity of the Phadiatop® assay in predicting the development of allergic post-race RTS was examined in 16 subjects.

CHAPTER 2 – LITERATURE REVIEW

2.1 Background

Athletes have a higher prevalence of respiratory symptoms than the general population (Hemingson *et al.,* 2004; Verges *et al.,* 2005) and upper respiratory tract infection (URTI) is the most common presenting complaint in sports medicine practices (Bermon, 2007). URTI interfere with training schedules and impair performance (Gleeson, 2007). While it appears that moderate intensity exercise may reduce chances of contracting URTI over sedentary controls, increased concentrations of cortisol and catecholamines released during high intensity, prolonged exercise may have a perturbing effect on the immune system. This has lead to the proposal of a 'J shaped curve' where moderate exercise is associated with reduced risk of URTI and either no exercise or high exercise loads with increased risk (Nieman, 2003; Moreira *et al.,* 2009).

It has been determined that high intensity prolonged exercise results in increased plasma concentrations of IL-6, IL-10 and IL-1ra suggesting a shift in dominance of T helper cell type 2 (T_H2) over T helper cell type 1 (T_H1) cells (Gleeson, 2007). It is believed that the more dominant T_H2 immune response after exercise, may up-regulate the actions of the humoral and allergic components of the immune system, and suppress the actions of the T_H1 mediated, cellular immunity. This transient suppression of the T_H1 cellular immunity has been implicated in the increased incidence of URTI infections in athletes after strenuous training or events (Peters, 2004; Bonini *et al.,* 2006) and an "open window" of opportunity for infection of 3 to 72hrs post exercise has been described (Peters, 2004). Peters *et al.* (1993) demonstrated a significantly increased incidence of self reported URTI symptoms in a double blind placebo controlled study in a large group of runners after an ultra marathon.

However, more recently, Spence *et al.* (2007) demonstrated that, although athletes displayed more symptoms of upper respiratory infection after competition or heavy training compared to controls (supporting the J shaped curve theory), pathogens were only isolated in 30% of cases. This has caused exercise immunologists to question whether other inflammatory or allergic

responses may be the cause of the increased reports of respiratory tract symptoms (RTS) noted after prolonged exercise (Peters, 2004; Schwellnus *et al.,* 2010). This uncertainty as to the exact cause of these symptoms has, more recently, lead to the terms Upper Respiratory Symptom (URS) or Respiratory Tract Symptoms (RTS) becoming the preferred terminology (Schwellnus *et al.,* 2010; Walsh *et al.,* 2010).

The current state of the knowledge regarding the three main theories proposed to explain the development of exercise-associated RTS (namely infectious, inflammatory and allergic) will now be discussed further.

2.2 Infection

Numerous studies have demonstrated increased incidence of URTI after exhaustive exercise (Peters and Bateman, 1983; Nieman *et al.,* 1989; Peters *et al.,* 1993) and this increased incidence has also been associated with periods of increased training load (Schwellnus *et al.,* 2010). The majority of the studies to date have used self reported symptoms and due to high costs of laboratory tests, the delay in receiving test results and the fact that physicians are rarely present in a research setting, very few of these studies verified the presence of infection (Walsh *et al.,* 2010). Thus it is more correct to talk of URS or RTS where infection has not been confirmed.

Furthermore, sports physicians are constantly endeavoring to differentiate between Upper Respiratory Tract Symptoms (URTS) including symptoms of the nose and pharynx, Lower Respiratory Tract Symptoms (LRTS) including chest symptoms and Systemic Symptoms (SS) including fever, myalgia, fatigue and headache (Schwellnus *et al.,* 2010). Although URTI or 'sore throats' are the most frequent reason for athletes to visit the sports physician, most RTS reported in exercise immunology studies are of short duration (1 - 3days) and do not involve SS (Walsh *et al.,* 2010) indicating that these are most likely not the result of a *de novo* infection.

The incidence of RTS in elite athletes do not necessarily follow the expected seasonal pattern noted in the general population, but rather centre around high intensity training in some sports (e.g. swimming) or after competitions (e.g. endurance running) (Schwellnus *et al.,* 2010). Other more illness-prone athletes may also experience RTS during routine training periods or following increases in training schedules (Walsh *et al.,* 2010).

Confirming the findings of Spence *et al.* (2007), Cox *et al.* (2008) identified organisms in 30% of cases and categorized a further 27% as suggestive of infection, but with no identifiable organisms. In conjunction with the findings of Reid *et al.* (2004) these studies also demonstrated that most positively identified infections are viral with only 5% of episodes caused by bacteria and that the causative organisms are those commonly associated with URTI in the general population. These studies reported approximately one third of cases of RTS caused by infection, another third caused by a non-infectious/ inflammatory medical cause and the final third unknown etiology. Furthermore, the diagnosis of infection by a sports physician (previously considered the gold standard) has been called into question and may not be the definitive test in determining RTS of infectious origin (Cox *et al.,* 2008).

However the commonly accepted hypothesis of an exercise induced immunosuppession still persists. This is based on the findings of numerous studies displaying the perturbing effect of an acute bout of strenuous exercise on various immunological parameters (Gleeson, 2007). This is most likely as a response to the increased concentrations of stress hormones during exercise. Following exercise circulating lymphocyte counts are depressed below pre-exercise levels for a number of hours (Gleeson, 2007) and natural killer cell counts drops to less than half of pre-exercise values (Shephard and Shek, 1999). Furthermore neutrophils display a diminished response to bacterial stimulation and lymphocytes' proliferative response to bacteria is diminished after an acute bout of exercise (Gleeson, 2007).

The cytokine response to an acute bout of exhaustive exercise is well studied (Walsh *et al.,* 2010). Cytokines are messenger chemicals found in plasma that are known to influence leukocyte function. Exercise causes a shift in cytokine concentrations that is very similar to tissue trauma, burns or infection (Smith, 2004). Typically exercise results in increases in

Interleukin- 6 (IL-6), IL-10, IL-1ra and Tumour Necrosis Factor Alpha (TNF α), whereas IL-2 and Interferon Gamma (INFγ) are down regulated (Gleeson, 2007). The release of these cytokines may be triggered by leakage of endotoxins from the intestines during exercise, elevation in circulating hormones (catecholamines and cortisol), raised body temperature, muscle damage, oxidative stress and glycogen deficiency (Nieman *et al.*, 2005). It has been noted that the particular pattern of cytokine imbalance is suggestive of a T_H2 lymphocyte dominant response to this type of stress (Lakier Smith, 2003; Peters, 2004). T_H2 lymphocytes secrete IL-4, IL-5, IL-6, IL-10, IL-13 and TNF α, whereas T_H1 lymphocytes secrete IL-2, IL-12, INFγ and TNFß (Smith, 2003). T_H1 lymphocytes are involved in cell-mediated immunity, while T_H2 lymphocytes are linked to humoral immunity. Therefore, this particular pattern of up regulation of T_H2 cytokines and low or undetectable levels of T_H1 cytokines is believed to indicate a transient suppression of cellular immunity by humoral immunity (Gleeson, 2007). Additionally, the percentage of T_H1 lymphocytes in circulation has been shown to decrease whereas the T_H2 lymphocytes remain the same (Lancaster *et al.*, 2004). These changes are most probably as a result of inflammation or increased cortisol concentrations. The above argument was previously used to support the infectious theory and to explain what was perceived as an 'open window' for infection (increased incidence of URTI symptoms) 3 – 72 hours post exercise.

However, recent reviews have found no significant link between changes in any single immune parameter (other than salivary IgA (sIgA)) and risk of RTS (Schwellnus *et al.*, 2010, Walsh *et al.*, 2010) and due to the complexity of the immune system, exercise immunologists have over the past few decades experienced difficulty in finding a single, reliable outcome measure to successfully measure immune suppression or susceptibility to disease. The only immunological parameter that has demonstrated a consistent relationship with RTS in athletes is concentration and secretion rate of sIgA which is commonly used as a marker of mucosal (or first line) immunity (Walsh *et al.*, 2010). The effect of exercise on secretion rate and concentration of SIgA is dependent on exercise intensity, with prolonged, high intensity exercise typically resulting in decreases in sIgA and shorter, lower intensity exercise causing moderate increases in SIgA. Walsh *et al.* (2010) state that there is a consistent association between decreased concentration or secretion rates of SIgA and increased risk of RTS or URTI in elite athletes. While Neville *et al.* (2008) determined in a large 50 week prospective study, that a decrease in

sIgA concentration of more than 40% from healthy baseline resulted in a one in two chance of developing an upper respiratory infection within the next 3 weeks.

A small proportion of elite athletes develop a syndrome consisting of repeated (frequent) RTS, chronic fatigue and poor performance. Reid *et al.* (2004) when studying a group from this population, displayed a high percentage of reactivation of Epstein Barr Viral (EBV) infections, and Gleeson *et al.* (2002) also demonstrated reactivation and a significant relationship between EBV sero-positive status and development of RTS. This has lead to the development of the viral reactivation theory for the aetiology of post-exercise RTS (Walsh *et al.*, 2010), this is supported by the fact that many RTS are of short duration and therefore not new infections, but rather related to chronic unresolved viral infection that may be reactivated by the stress of exercise (Reid *et al.*, 2004).

2.3 Inflammation

Under normal conditions, the airways condition inspired air, filtering, warming and humidifying it, thereby protecting the lower airway. This process is dependent on the nasal mucosal blood vessels and glands, which are under autonomic control (Bonini *et al.*, 2006; Swartz *et al.*, 2008). Hyperpnoea resulting from prolonged exercise results in water loss from respiratory mucous membranes, which in turn puts the stress of conditioning the air onto the smaller bronchioles. This results in a 'dehydration injury', which causes a type of airway sensitivity called hyper-reactive airways (HRA) (Anderson and Kippelen, 2008). Athletes are particularly susceptible to this mucosal drying, which occurs regularly over a training season, and results in airway changes such as increased numbers of neutrophils, eosinophils and lymphocytes noted at rest, demonstrating a lingering or chronic inflammatory process (Bermon, 2007; Anderson and Kippelen, 2008). HRA is aggravated by cold dry air and chronic inhalation of allergens and/or irritant particulate matter, and hence certain sports exposed to these factors show higher levels of sensitive individuals. These sports include swimmers (exposed to chlorine), endurance runners (allergens and pollutants), and cross country skiers (cold air inhalation) (Rundell and Jenkinson,

2002; Bermon, 2007). The pathogenesis of this hypersensitivity is believed to start with dehydration injury and changes in the osmolarity of the airway cells. The resultant influx of water to correct this imbalance is believed to stimulate the release of inflammatory mediators, potentially stimulating bronchoconstriction, vasopermeability and mucus hypersecretion (Bermon, 2007; Swartz *et al.,* 2008). It is believed that, as water loss from the airways is common to all who exercise, the ability to return moisture to the dehydrated mucosal surface must be different between healthy and inflamed airways (Andersen and Kippelen, 2008). An alternative explanation for HRA states that temperature changes stimulate bronchial airway narrowing and that at the end of the exercise the rapid re-warming results in a reactive hyperemia that causes further bronchospasm (Rundell, 2002). It is likely that mast cells are important in the pathogenesis of hypersensitivity. Mast cells release bronchoconstrictor mediators such as histamine, cysteinyl leukotrines and prostaglandins following exercise (Carlsen *et al.,* 2008). Sensitized airway smooth muscle may be infiltrated with mast cells and increased numbers of mast cells have been found in the airway smooth muscle of both asthmatic and healthy athletes (Anderson and Kippellen, 2008).

Many studies show increased numbers of inflammatory cells in broncho-alveolar lavage fluid of endurance athletes (Bermon, 2007). For example increased T-lymphocytes, neutrophils, eosinophils and macrophages in cross-country skiers, runners show increased neutrophilia, swimmers show neutrophilia but no eosinophilia (Bermon, 2007). Rong *et al.* (2008) demonstrated significantly higher levels of neutrophils and macrophages in the saliva of swimmers at rest, compared to controls, indicating airway inflammation. However findings have not always been consistent, with some studies displaying neutrophilia without eosinophilia and vice versa (Parsons *et al.,* 2008). It should be noted that healthy control subjects rarely exhibit sputum eosinophilia (Helenius *et al.,* 2005). In spite of the elevated numbers of inflammatory cells, markers of inflammation such as eosinophil peroxidase and neutrophil lipocain are generally normal (Bermon, 2007). As neutrophils are attracted to the airway by inflammatory mediators (IL-8, IL-1B, TNF-a, leucotrines etc.) (Bush, 2010), it is believed that this increased cellularity could represent systemic as opposed to local inflammation, as an absence of local neutrophil activation has been noted along with decreased expression of eosinophil and macrophage surface adhesion molecules. Therefore, the concept of “blunted” or “frustrated”

inflammation has been suggested (Bermon, 2007). However it may be possible that under certain conditions, a loss of control of this inflammation may occur, resulting in an acute airway inflammation causing similar symptoms to URTI. Cox *et al.* (2007) when comparing the post exercise inflammatory responses of healthy and illness prone athletes found that the illness prone athletes displayed an altered state of inflammatory control and a potential for excessive inflammation. Furthermore, structural changes such as lymphoid aggregates and basal membrane changes have also been observed on bronchial biopsies of cross country skiers (Bermon, 2007).

However, experiments such as those of Cox *et al.* (2010) using local anti-inflammatory agents (Difflam throat spray) are open to interpretation. The incidence of RTS events was not reduced but the severity of symptoms was lessened by the intervention suggesting that the reaction does not just involve local factors but rather systemic inflammatory markers (Cox *et al.,* 2010).

2.4 Other non-infectious causes

2.4.1. Airway Hypersensitivity

There are numerous factors that contribute to hypersensitivity in the airways and these hypersensitive states can lead to a sensitization or respiratory hyper responsiveness to additional stimuli, such as exercise. This could potentially produce RTS or conditions such as hyper-reactive airways (HRA) or exercise induced bronchospasm (EIB), a form of non-allergic asthma. Aetiological factors include chronic or repeated exposure to cold or dry air as seen in cross-country skiers (Langdeau *et al.,* 2000; Hemingson *et al.,* 2004), exposure to allergens, for example in endurance runners (Bonini *et al.,* 2006; Schwellnus *et al.,* 2010) and exposure to various pollutant irritants, which may affect a number of different sports people. Examples of pollutant irritants include chlorine, which may explain the high incidence of upper and lower airway symptoms, EIB and asthma in elite swimmers (Langdeau *et al.,* 2000). Sulphur dioxide, from the breakdown of fossil fuels (smog). When dissolved in the surface fluids of the lung sulphur dioxide yields sulphuric acid and other irritants, which damage epithelium and stimulate the release of inflammatory mediators. This may contribute to wheeze, tight chest, cough and

sputum in HRA or asthmatic subjects (Bonini *et al.,* 2006). Particulate matter are small particles in the air (< 10μm) that are deposited in the lower airways, these acidic particles may aggravate respiratory symptoms and decrease lung function by activating leukotrines and stimulating inflammation (Rundell *et al.,* 2003; Bonini *et al.,* 2006). Particulate matter may also act as an attractant for neutrophils (Andersen and Kippelen, 2008). Chronic exposure to ozone is also known to cause respiratory symptoms and decreases in lung function. Ozone may also result in transient airway hyper-responsiveness, and nasal and lung allergen responsiveness is increased by ozone. Nitrogen dioxide from motor vehicles does not affect healthy individuals but in asthmatics this enhances airway responsiveness (Bonini *et al.,* 2006). Thus certain sports might put people at risk of chronic exposure to these irritants and increase the possibility of airway hyper reactivity or inflammation. Research has indicated a gender difference in HRA in athletes, with women showing a significantly higher prevalence than men. It is hypothesized that this may be due to the smaller diameter of women's airways (Langdeau *et al.,* 2009). Carlsen, (2009) found that the incidence of bronchial hyper-responsiveness (BHR) increased with age and duration of training. This finding was also confirmed by Pedersen *et al.* (2009) in their study on HRA in adolescent swimmers. These factors are also believed to contribute to the development of non-allergic rhinitis (NAR) a condition that mimics allergic rhinitis in symptomatology, but presents with normal serum (Bousquet *et al.,* 2008).

Under normal circumstances exercise improves nasal efficiency and results in a reduction of nasal resistance of up to 50% due to an increase in nasal sympathetic tone and vasoconstriction (Bonini *et al.,* 2006). However, cold air induces glandular secretion and nasal discharge in normal subjects, a response that is exaggerated in rhinitics. Swimmers are prone to a chemical sensitivity known as 'chloride treated water allergy' characterized by nasal obstruction, watery nasal discharge and sneezing (Bonini *et al.,* 2006) which may predispose swimmers to rhino-sinusitis, rhino-otitis and asthma. Divers are prone to barometric rhino-sinusitis due to repeated sudden pressure changes (Bonini *et al.,* 2006). Another non-allergic type of rhinitis is seen in cold weather endurance athletes and is commonly known as 'skier's nose'. This vasomotor / non-allergic rhinitis occurs when cold temperatures trigger a parasympathetic reflex resulting initially in nasal discharge and later in nasal congestion due to the dilation of the turbinate

vessels and increased mucus transport times (Bonini *et al.,* 2006). Finally, some predisposed individuals appear to experience excessive drying of the rhinosinusal mucosa in response to prolonged exercise. This leads to thickening of nasal secretions and increased muco-cillary transport times, and also promotes a rebound increase in nasal secretion and repeated bouts of watery rhinorrhea commonly called 'runners nose' (Bonini *et al.,* 2006). It is conceivable that the mucus membrane damage caused by thermal, osmotic or irritant stimuli, and its resulting inflammation, may sensitize the individual and increase the likelihood of developing further IgE mediated allergies.

Lower airway hypersensitivity is evident in cold weather athletes where it is commonly known as 'ski asthma'. Symptoms include coughing, wheezing tight chest, dyspnoea and excessive mucus production (Rundell and Jenkinson, 2002). It is believed that oedema and excessive mucus production could amplify the bronchoconstriction seen in cold weather athletes and account for the pathogenesis of 'ski asthma' and the increased incidence of respiratory symptoms seen in these athletes (Anderson and Kippelen, 2008, Carlsen *et al.,* 2008). Thus the airway hyper-responsiveness evident here would be the result of airway injury rather than a sign of classic asthma (Bonini and Craig, 2008).

Exercise induced bronchospasm (EIB) is the term used to denote the airway hyper-reactivity observed in non-asthmatic, non-atopic populations (Rundell and Jenkinson, 2002). Although bronchoconstriction is common in asthmatics EIB affects the athletic population as a whole, with research showing a prevalence of 50% in athletes (Rundell and Slee, 2008; Parsons *et al.,* 2008). Parsons *et al.* (2008) demonstrated increased concentrations of inflammatory mediators in the airways (sputum) of non-asthmatic athletes who experienced EIB after exercise, this finding that was confirmed in the research of Verges *et al.* (2005). Significant differences in concentrations of cystinal-leucotrines, leucotrine B4, thromboxane B2, and prostaglandin E2 were noted in athletes after eucapnic voluntary hyperventilation to induce EIB (Parsons *et al.,* 2008). Neutrophlia in the lower airways of endurance athletes may be the result of airway injury, or due to the increased incidence of viral infections in this population. In athletes exposed to viral infections, allergens, irritants and particulate matter, there may be activation of other innate immune mechanisms rather than IgE. Therefore this may be a type of 'neutrophilic' as opposed

to eosinophilic asthma (Anderon and Kippelen, 2008). It has also been demonstrated that high intensity exercise results in increased markers of pulmonary epithelial permeability. This disruption of the epithelial barrier has been implicated in the development of asthma in athletes as a result of exposure to pulmonary irritants (Anderson and Kippelen, 2008). Finally injury to the epithelium may expose sensory nerve endings to foreign particle or endogenous inflammatory mediators. It is hypothesized that EIB in cold weather athletes could be partly triggered by neuropeptide from stimulated sensory nerves (Anderson and Kippelen, 2008) and therefore may be neurological rather than purely inflammatory.

2.4.2. Allergy

Allergy has also been proposed as a possible cause of non-infective RTS. In fact, the exercise-induced T_H2 / T_H1 dominance cytokine pattern is one now commonly associated with the development of allergies and asthma. Allergies are typically associated with raised IL-4, IL-5, IL-10, and IL-13 and low INFγ, and are also associated with high serum IgE (sIgE) concentrations (Meischer and Vogel, 2002; Neaville *et al.*, 2003). Rong *et al.* (2008) demonstrated raised serum concentrations of IL-4 and lower concentrations of IL-10 in a population of Chinese professional athletes when compared with controls. Swimmers and endurance athletes demonstrated the highest concentrations of IL-4, as well as the greatest indices of small airway dysfunction. Il-4 is a T_H2 cytokine and is known to up-regulate the IgE receptors on B lymphocytes, hence contributing to the inflammatory response resulting in asthma. Il-10 exerts an opposite anti-inflammatory effect. This finding was supported by an earlier animal study by Davis *et al.* (2005), who demonstrated that cold air exercise in horses produced a predominantly T_H2 cell dominant pattern of cytokine production in bronchoalveolar lavage fluid. Significantly higher concentration of IL-4, IL-5 and IL-10 were noted, along with a lesser up-regulation of IL-2 and IL-6. It was proposed that this shift was a result of mast cell activation due to airway cooling and desiccation, and hypothesized that this mechanism may be responsible for the development of asthma.

The T_H2 dominant shift has also been associated with a higher incidence of allergies in athletes (Bonini and Craig, 2008). Certainly, it has been demonstrated that atopy is a major risk factor for HRA, EIB and exercise induced asthma (EIA) in athletes, and it has even been suggested that atopy is more common among athletes (Helenius *et al.*, 1998; Langdeau *et al.*, 2000). Kyllonnen *et al.* (2006) demonstrated that patients with atopic dermatitis produced more respiratory symptoms, HRA and sputum eosinophilia than controls. It has also been suggested that airway injury and increased mucosal permeability may promote the development of asthma, particularly in predisposed (atopic) individuals (Anderson and Kippelen, 2008) and that repeated exposure to damaging pollutants increases the potential for allergic responses in the predisposed (Bonini *et al.*, 2006). This is supported by the findings of Aldred *et al.* (2010) who demonstrated a significant elevating effect of steady state exercise on sIgE concentrations of individuals with prediagnosed allergies.

Eosinophilia is a characteristic of most allergic disorders (Morris, 2009). T cell mediated eosinophilia is mostly driven by T_H2 T cells, with IL-5 being the most important cytokine for differentiation and activation of eosinophils (Simon and Simon, 2007). It is possible certain eosinophilic, allergic reactions may be triggered by IL-5 releasing T cells being stimulated by atypical means e.g. infective or autoimmune. Additionally, a subgroup of allergic rhino conjunctivitis, asthma and dermatitis (known as 'intrinsic') are not driven by IgE mediated pathways, although the eosinophilia seems to be similar and also driven by IL-5 releasing T_H2 cells (Simon and Simon, 2007).

It has been documented that athletes experience higher rates of allergic diseases than the general population (Thomas *et al.*, 2010) and that the cited incidence of allergy among Olympic athletes is steadily increasing (Weiler *et al.*, 1998; Katelaris *et al.*, 2003; Bonini *et al.*, 2006). As allergic reactions can produce a range of upper and lower respiratory symptoms that closely mimic URTI (Schwellnus *et al.*, 2010) and as exercise is known to induce a T_H2 dominant immunological shift, it is proposed that exercise may up-regulate an allergic response in those already sensitised (Lakier Smith, 2003), and an increased exposure of athletes to irritants and allergens (Bonini *et al.*, 2006; Schwellnus *et al.*, 2010) may contribute towards this. Recently, Schwellnus *et al.*

(2011) related the high incidence of pre-race RTS symptoms documented in triathletes to allergy rather than any other factor.

Although the skin prick test (SPT) is generally accepted as the standard method for detecting IgE related allergic sensitization (Vidal *et al.*, 2005) and is cheaper, faster and has less false positives than specific IgE antibody testing used in the Phadiatop® assay (Morris, 2009) it does have certain limitations. These include lower response in the elderly, greater difficulty in grading the response in darkly pigmented persons, contraindication for the pregnant, the quality and selection of allergens, theoretical risk of anaphylaxis and operator inexperience (Antunes *et al.*, 2009; Morris, 2009) which may hinder its application.

Specific IgE antibody testing is accepted as an alternative to the SPT and combination tests exist, such as Phadiatop® assay (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) which simultaneously test for IgE to a mixture of allergens causing common inhalant allergies. Allergens included in the Phadiatop® assay are Artemisia, dust mites (D. pteronyssinus and D. fariane), mixed moulds (Penicillium, Cladosporium, Aspergillus and Alternaria), pet dander (cat and dog), mixed grasses (Parietaria, Lolium, Phleum and Cynodon), and mixed trees (Acer, Betula, Olea, Salix, Pinus, Ulmus, Quercus, Eucalyptus, Acaciaand Malaleuca) (Garcia-Marcos, 2007).

The Phadiatop® assay has not only been found to offer satisfactory accuracy for diagnosing of IgE allergic sensitization in the general population (Vidal *et al.*, 2005), but has also been used previously in exercise immunology as part of an assessment of atopy in athletes (Moreira *et al.*, 2007) during the Helsinki city marathon. Vidal *et al.* (2005) found a sensitivity of 70.8% and a specificity of 90.7% compared to the SPT. They also displayed a 6.9% false positive rate and a 7.5% false negative rate compared to SPT. Garcia-Marcos *et al.* (2007) displayed a rate of false positives of 10% and found that the lower the rate of atopy in a population the higher the rate of false positives. However, the allergens included in the Phadiatop® assay are fixed and cannot be varied from area to area, the test is more expensive than SPT and the results take longer to be processed.

Allergic rhinitis occurs commonly in athletes, with 16.8% of athletes suffering from hay fever (Bonini *et al.*, 2006). Rhinitis and asthma are connected, with allergic rhinitis increasing the risk of other airway symptoms. The link between allergic rhinitis and asthma is further reinforced by the fact that controlling rhinitis leads to better control of asthma (Serrano *et al.*, 2004; Bonini *et al.*, 2006; Schwartz, 2008). It is also believed that the incidence of allergic rhinitis is increasing, in 1980, 8% of Olympic athletes were found to have clinical rhinitis and the figure had doubled (16.9%) by 1996 (Bonini *et al.*, 2006). Another study found that of 214 athletes from 12 Olympic sports disciplines, 56% suffered from allergic rhino conjunctivitis and 41% had both symptoms and a positive skin prick test (Carlsen *et al.*, 2008). Nasal inflammation in allergic rhinitis is accompanied by a significant increase in eosinophils in the epithelium and nasal lamina propria, as well as increased IL-5 and other inflammatory cytokines in the sputum (Serrano *et al.*, 2005). Allergic rhinitis has been shown to negatively affect the ability of athletes to train and compete, and athletes on treatment with intranasal steroids showed increased performance scores (Bonini *et al.*, 2006). Carlsen *et al.* (2008) report that the cited rate of allergic rhinitis in athletes is clearly higher than in the normal population.

Asthma is defined as a chronic inflammatory disease of the respiratory tract in which a number of cells play a role including mast cells, eosinophils and T Lymphocytes. It leads to increased HRA, dyspnoea, wheezing, cough and shortness of breath (Bonini *et al.*, 2006). EIB and exercise induced asthma (EIA) are similar, but EIB is a term used in non-asthmatics and EIA is a term reserved for broncho-constriction in asthmatics (Carlsen *et al.*, 2008). Not all asthmatic subjects experience EIA, it appears to be a common, but discrete clinical phenotype (Hallstrand *et al.*, 2005) that shows increased concentrations of epithelial cells, eosinophils, cysteinyl leukotrines and decreased PGE2 in sputum compared to non EIA asthmatics (Hallstrand *et al.*, 2005). The similarities between these findings and the findings of other studies on non-asthmatic EIB positive athletes are interesting and raises the likelihood of chronic exercise induced HRA, leading to EIB and eventually possibly resulting in asthma (Davis *et al.*, 2005). HRA and asthma are significantly more common in cross country skiers when compared to healthy control subjects (Helenius *et al.*, 1998; Davis *et al.*, 2005). The occurrence of asthma in elite athletes is on the increase, statistics indicated that 9.7% of athletes in the 1976 Olympics were asthmatic, this increased to 21.9% of athletes in 2000 Sydney Olympics (Bonini *et al.*,

2006; Carlsen *et al.,* 2008). Asthma is also more common in elite athletes than in age matched controls (Bonini *et al.,* 2006). Asthma and allergic rhinitis often co-exist, with 80-90% of asthmatics suffering from rhinitis and 19-38% of rhinitics suffering from asthma (Serrano *et al.,* 2004; Bonini *et al.,* 2006).

Atopy is a major risk factor, together with the type of training, for an athlete developing asthma (Helenius *et al.,* 1998). The relative risk of developing asthma is increased by 25 times in atopic speed and power athletes, and increased by 75 times in atopic endurance athletes, when compared to non-atopic controls (Schwartz *et al.,* 2008). Mild asthma (when defined as bronchial hyper-responsiveness (BHR) to histamine or metacholine challenge) is most common in endurance athletes, with the highest numbers prevalent among cross country skiers (14.4 - 54.8%), swimmers (13.4% - 44%), and long distance runners (14.8% - 19%) and track and field athletes (16.3%) (Helenius *et al.,* 2005). It is believed that the remodeling seen in the basement membranes of athletes with mild asthma could diminish their capacity to respond to evaporative water loss, and therefore increase susceptibility to dehydration injury (Rundell and Jenkinson, 2002).

The chronic eosinophilic inflammation seen in the lower airways of asthmatics may lead to airway remodeling such as epithelial desquamation, loss of tight junctions, metaplasia of epithelial goblet cells, thickening of the reticular basement membrane, hypertrophy of airway smooth muscle, fibrosis of the subepithelium, an increase in mucus glands as well as increased angiogenesis (Tsurikisawa *et al.,* 2010). The extent of the remodeling has been linked to the duration and severity of the asthma and the serum levels of IgE (Tsurikisawa *et al.,* 2010). Airway remodeling has also been linked to BHR and long term decline in lung functions of adult asthmatics (Tsurikisawa *et al.,* 2010). Another interesting finding that may be relevant to asthmatic athletes is the relationship between neutrophilic inflammation in the airways of stable asthmatics and a lack of airway hydration, as demonstrated by Loughlin *et al.* (2009). Due to the fact that airway dehydration has been implicated in airway injury and HRA, asthmatic athletes may be more susceptible if their airways are already less hydrated. It has also been suggested that this airway dehydration may be the cause of the bronchoconstriction and mucus build-up (decreased muco-cillary clearance) that is central to asthma (Laughlin *et al.,* 2009).

2.4.3. Non-allergic Rhinitis

Non-allergic rhinitis (NAR) is a condition characterized by symptoms similar to allergic rhinitis, such as anterior or posterior nasal discharge, sneezing, nasal obstruction and irritation, but presents with normal serum IgE levels and a negative skin prick test or Phadiatop® test for atopy (Wedback *et al.*, 2005). The prevalence in the adult population is believed to be at least 25% (Bousquet *et al.*, 2008). Although the exact cause is unknown the underlying mechanisms that have been postulated include environmental triggers (irritant pollutants); persistent inflammatory mechanisms; auto-immune dysregulation and neurogenic mechanisms (Bousquet *et al.*, 2008). The airways of athletes may be exposed to a variety of increased stressors that may predispose them to this type of disorder. Repeated dehydration injury, irritant pollutant and allergen exposure, cortisol and catecholamine fluctuations and temperature extremes may all contribute to the development of such well known, non-allergic and exercise related entities as “skiers nose”, “runners nose” and “chloride treated water allergy” (Bonini *et al.*, 2008). The diagnosis of NAR is based on the exclusion of criteria such as allergy and infection, rather than on condition specific inclusion criteria (Bousquet *et al.*, 2008).

2.5. Conclusion

Therefore, there is sufficient evidence to indicate that not all cases of post-exercise RTS are the result of infection, and while numerous studies provide evidence for an inflammatory aetiology, in a large percentage of cases the cause remains unknown. Although certain non-infectious mechanisms have been proposed and while the incidence of allergic diseases in athletes is increasingly well documented, the relationship between allergic reaction and the development of post-exercise RTS has not been well explored. Specifically, the contribution of allergic reaction to the incidence of post-exercise RTS in trail-runners in a rural environment is an area where little work has been done to date.

CHAPTER 3 – SCIENTIFIC PUBLICATION 1

Upper respiratory tract symptoms in multi-day trail runners not linked to allergy alone

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Key Words: Respiratory tract symptoms, trail runners, allergy, *de novo* infections, reactivation of prior infections, inflammation.

Short running header: Upper respiratory tract infections in trail runners

ABSTRACT

Objectives: To document the incidence of upper respiratory tract symptoms (URS) for two weeks before and two weeks after a three-day trail run and relate these to the general systemic and salivary immunological profile as well as atopic status of the participants.

Study Design and Methods: A descriptive field study was conducted at the Three Cranes Challenge Trail run, a multi-day 95 km event divided into three stages, in Karkloof, KwaZulu-Natal. Fourteen runners were monitored over a 31 day period before and after the race. Main outcome measures included the incidence of URS, Phadiatop® status, changes in concentrations of serum IgE, cortisol, high sensitivity C - reactive protein (hs-CRP), differential leukocyte count and sIgA.

Results: Eleven of 14 subjects (78.6 %) met the criteria for positive diagnosis of URS during the 14 days post-race. In four of the symptomatic subjects (36.4 %), URS appeared to be of inflammatory origin, but these were not linked to systemic markers of an allergic response. Six (54.5 %) of these symptomatic subjects presented with markers of infection, three (27.3 %) with markers of a *de novo* infection and three (27.3%) with a profile suggestive of reactivation of previous infection. Four (66.7 %) of those presenting with markers of infection had concomitantly elevated levels of serum IgE suggestive of allergic response. There was no evidence of the isolated presence of an allergic reaction among the symptomatic subjects.

Conclusion: URS in trail runners has a multifactorial aetiology. A link between concurrent markers of an allergic response and infection is common in symptomatic trail runners.

INTRODUCTION

It is well documented that athletes have a higher prevalence of respiratory symptoms than the general population.[1,2] with upper respiratory tract infection (URTI) being the most common presenting complaint among elite athletes in sports medicine practices.[3] Although epidemiological studies have confirmed increased incidence of self reported URTI symptoms after exhaustive exercise,[4] few of these symptoms are likely to have been caused by infection.[5] Clinical studies have reported that approximately one third of cases of URTI symptoms are caused by infection[6-8] with another third caused by a non-infectious medical conditions and the final third of unknown aetiology.[5] This has lead to the preferred current use of the broader term, upper respiratory symptoms (URS).[5]

A number of hypotheses have arisen in an attempt to explain the development of non-infectious URS such as an inflammatory response,[3] reactivation of prior infection[8] and airway hypersensitivity.[9,10] The cytokine response to prolonged exercise has been hypothesised to result in an alteration in immune function which may predispose an individual to the development of allergies.[11] More recently, an actual association between responsiveness to allergens and the development of URS in athletes, has been proposed.[12]

Furthermore, contributors to variants of non-allergic rhinitis (NAR) which involve concomitant hypersensitivity and inflammation in the airways, include chronic or repeated exposure to dry air[1], prolonged hyperventilation, increased environmental exposure to inhaled allergens such as pollens[13] or various pollutant irritants which could stimulate the release of inflammatory mediators and result in hyper reactive airways (HRA) in endurance runners.[9,13] Although airway changes such as increased numbers of neutrophils, eosinophils and lymphocytes, demonstrating a lingering or chronic inflammatory process,[3,9] have been reported at rest, markers of activation of these immune cells are typically lacking, leading to the description of a state of 'blunted'[3] or 'frustrated'[10] inflammation. These conditions which mimic allergic rhinitis, present with normal serum IgE levels.[13]

There is therefore still much debate regarding the aetiology of a large percentage of post exercise

URS and the possible interactions between infectious, inflammatory and allergic origins remain unexplored. As a clearer understanding of the underlying factors may allow effective preventative strategies to be developed, the overall aim of this study was to conduct individual case studies describing the general immunological profile of 14 athletes taking part in a three-day 95 km trail running event and relate these to the self-reported incidence of URS. The specific aims were to i) determine the atopic status of each subject, ii) measure the concentrations of serum IgE, cortisol, high sensitivity C- Reactive Protein (hs-CRP) as well as differential leukocyte and secretory immunoglobulin A (sIgA) concentration throughout and after the event and iii) to relate afore-mentioned to the incidence of self reported URS pre, during and post event.

METHODS

Approval to conduct this research was obtained from the Bioethics Committee of the University of KwaZulu-Natal. This study formed part of a larger, more comprehensive work examining physiological responses of athletes who had registered for the Three Cranes 95km three-day trail run in Karkloof, KwaZulu-Natal which involved completion of 27.8, 37.9 and 29.3 km on days 1, 2 and 3, respectively.

A convenience sample of 22 (7 male, 15 female) volunteers between the ages of 25 and 50 years, were asked to sign an informed consent form (Appendix B). In order to qualify for this study, runners needed to complete all three stages of the race and be free of any medical condition that could place their health at risk. Further exclusion criteria included smoking, the use of oral or inhaled anti-histamines or corticosteroids and the use of performance enhancing drugs.

Baseline assessments included basic anthropometric measurements and vital signs at registration on the day before the race; blood sampling commenced the morning prior to the race. Blood samples were collected at eight time-points (Figure 1); before and after each day's stage, 24 hours post race and 72 hours post race. Saliva samples were collected between 60-90 minutes before the start of the race/Stage 1 ($S1_{pre}$), within 60 minutes following completion of the race/ Stage 3 ($S3_{post}$), 24 hours (24PR) and 72 hours post race (72PR). The athletes were also

requested to document their experience of URS over a 31 day period, viz. during the 14 days prior to the start of the race, on each day of the race and during two weeks following the race.

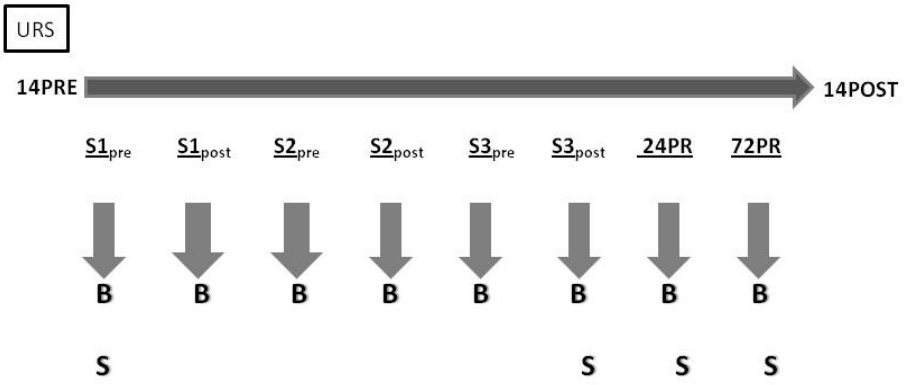

Figure 1. Time line of blood, saliva and URS sampling during the study
14PRE = 14 days prior to the race; $S1_{pre}$ = prior to the start of stage 1 of the race; $S1_{post}$ = immediately after finishing stage 1 of the race; $S2_{pre}$ = prior to the start of stage 2 of the race; $S2_{post}$ = immediately after finishing stage 2 of the race: $S3_{pre}$ = prior to the start of stage 3 of the race; $S3_{post}$ = immediately after finishing stage 3 of the race: 24PR = 24 hours after the finish of the race: 72PR = 72 hours after the finish of the race. 14POST = 14 days after the finish of the race; URS = upper respiratory symptom questionnaire; B = Blood Sample; S = Saliva Sample.

Of the 22 subjects who started the study, two subjects were excluded from the study due to injury and illness, and a further four were unable to attend the post race sampling sessions. Of the remaining 16 volunteers who completed all three stages of the race and complied with all protocol requirements, a further two were excluded due to the use of the anti-histamine medication.

Athletes were asked to record a detailed log of their self reported URS incidence and severity according to a 1 – 3 point scoring system over the two weeks prior to the start of the race

(Appendix C). During the race further information was elicited each day pre and post stage. A post race URS questionnaire was used to record the incidence and severity of any URS over the two-week period following the race. The severity-ratio of each symptom used in the questionnaires was calculated from the sum of the above mentioned severity scores, divided by the number of days the symptom presented. A global symptom severity score was then determined by multiplying the severity ratio for each symptom by the duration of the illness. In an attempt to exclude symptoms of a trivial nature or not specific to the URT, a single URS lasting < 2 days and any non-specific symptom not accompanied by a URT symptom lasting > 1 day, were omitted.

Saliva collection took place via passive drool on subjects in the seated position with their head tilted slightly forward and minimal orofacial movement. After swallowing the saliva collected during the first minute, saliva samples were collected for four minutes in pre-weighed polypropylene microvials. Weighed tubes were then placed in a freezer (- 20^{o}C) and later stored at - 80^{0}C until analysis. The saliva volume was calculated from pre- and post- mass difference and saliva flow rate (ml.min^{-1}) was determined by dividing this by the collection time.

Concentration of sIgA (µg IgA x ml^{-1}) was determined using an indirect enzyme immunoassay kit (Salimetrics, State College, USA). Salivary IgA secretion rate (µg sIgA x min^{-1}), or the total amount of IgA appearing on the mucosal surface per time unit, was calculated by multiplying sIgA concentration by saliva flow rate.

Venous blood samples were obtained at each of the eight testing periods from the antecubital vein of each subject in the seated position. Complete blood counts were measured on an Advia-120 Hematology Analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) at a commercial pathology laboratory (Ampath Laboratories, Howick). Extracted serum was immediately frozen in dry ice and transported to the laboratory for the determination of serum IgE, hs-CRP, cortisol and Phadiatop® assay. The latter, which measures concentrations of specific IgE antibodies towards a pre-determined selection of inhalant allergens[14] was run using an automated UniCAP system, according to the instructions of the manufacturers (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden).

Plasma volume (PV) changes were determined from pre- and post-exercise haematocrit and haemoglobin concentrations according to the method of Dill and Costill.[15] All concentration dependant, post stage blood cell parameters (IgE, leukocyte counts, cortisol, and hs-CRP values) were then adjusted for percentage change in PV.

Statistics

The majority of data are presented individually for each case study. In terms of the grouped data of the cohort (n=14), the abnormal distribution was confirmed using the Kolmogorov–Smirnov test and they are expressed as median (range). After logarithmic transformation, a generalized linear model was used on the grouped data for each parameter tested to identify any significant group or time effect. Statistical analysis was performed using SPSS version 18 (SPSS Inc., Chicago, USA) and significance was set at p=0.05.

RESULTS

The physical characteristics and results of baseline testing of the 14 subjects (10 female and 4 male) are shown in Table 1. Minimum temperature at the start of the stages ranged from 11.5º C (S1) - 12.4º C (S3), whereas maximum temperature ranged from 21.2 º C (S3) - 22.8 º C (S2). At no stage did the wind speed exceed 2.8 m/s nor did it rain. A maximum relative humidity of 97% was recorded during S2.

Table 1: Median (range) physical characteristics of the subjects (n=14).

Subjects (n=14)		
Variable	**Median**	**Range**
Age (years)	42.5	20.0 - 50.0
Stature (cm)	168.0	158.0 - 184.0
Mass (kg)	62.4	49.1 - 90.6
BMI	23.3	19.7 - 27.7
% Body Fat*	21.8	15.7 - 30.6
Resting Heart Rate	57.5	49.0 - 66.0
Resting Systolic BP	122.5	117.3 - 138.5
Resting Diastolic BP	81.9	72.0 - 93.3

*Derived from the sum of triceps, biceps, suprailiac and subscapular skin folds.[16]

The changes in median (range) of selected red blood cell indices reflect a drop in median (range) of haematocrit and haemoglobin concentrations and rise in percentage plasma volume (% PV) calculated for each stage over the course of the 3 day event and versus S1pre as baseline. Median (range) % PV increased by 4.40 (-10.9 - 16.4), 12.70 (-4.3 - 34.8) and 5.10 (-10.7 - 28.5) % during S1, S2 and S3, respectively. Table 2 presents the race – induced perturbations in total and differential leukocyte counts. These show increases above the upper limit of the clinical reference range[16] in the post stage total leukocyte and neutrophil concentrations only.

Table 2. Median (range) total leukocyte and differential white cell concentrations# for the subjects (n=14) at eight time points.

Time Period	Total leukocyte 10^{9}/L	Neutrophil 10^{9}/L	Lymphocyte 10^{9}/L	Eosinophil 10^{9}/L	Basophil 10^{9}/L
Reference Range[17]	3.92 - 9.88	2,.00 - 7.50	1.00 - 4.00	0.00 - 0.45	0.00 - 0.20
$S1_{pre}$	5.69 (4.32 - 7.72)	2.91 (1.1 - 4.3)	1.93 (1.38 - 3.32)	0.21 (.06 - 0.65)	0.035 (0.02 - 0.08)
$S1_{post}$	12.94* (7.90 - 16.00)	10.87* (6.1 - 14.6)	1.50 (0.51 - 2.20)	0.06* (0.01 - 0.26)	0.049 (0.02 - 0.10)
$S2_{pre}$	7.12 (4.53 - 10.10)	3.60 (1.7 - 6.0)	2.06 (1.43 - 3.70)	0.24 (0.06 - 0.72)	0.050 (0.01 - 0.08
$S2_{post}$	12.56** (8.22 - 19.68)	10.07** (5.7 - 15.2)	1.77 (1.11 - 2.92)	0.05** (0.00 - 0.38)	0.049 (0.02 - 0.25)
$S3_{pre}$	7.63 (6.09 - 10.86)	4.37 (1.8 - 5.6)	2.49 (1.91 - 4.42)	0.29 (0.1 - 0.73)	0.056 (0.03 - 0.08)
$S3_{post}$	11.05*** (6.79 - 17.87)	7.55*** (5.1 - 14.5)	1.64 (1.38 - 2.83)	0.07*** (0.01 - 0.37)	0.043 (0.02 - 0.07)
24PR	7.79 (4.61 - 10.18)	4.33 (2.3 - 5.4)	2.47 (1.46 - 4.24)	0.20 (0.07 - 0.63)	0.049 (0.00 - 0.08)
72PR	7.53 (3.91 - 10.13)	4.85 (1.7 - 6.5)	1.68 (0.72 - 3.49)	0.14 (0.02 - 0.39)	0.033 (0.02 - 0.06)

#Adjusted for plasma volume changes* $p<0.05$ vs. S1pre, ** $p<0.05$ vs. S2 pre *** $p<0.05$ vs. S3 pre (generalized linear model).

Phadiatop® test results, peak serum IgE concentrations and URS status of the individual subjects are presented in Table 3. Specific IgE antibody concentrations were above > 0.35 IU/ml in seven

subjects who were classified as Phadiatop positive,[17] while the remaining seven subjects did not respond to exposure to the inhalant allergens included in this test.[14] In 86% of subjects the peak IgE concentrations were recorded at the 24PR time point. Race-induced median (range) serum IgE concentrations of the subjects are presented in Figure 2. The overall rise over the three-day event, was not significant (p=0.37). Only four URS positive subjects presented with clinically elevated IgE concentrations.[17]

Table 3: Individual baseline pre-race Phadiatop® responses and peak serum IgE concentrations and URS status (n=14)

Case Number	Phadiatop® Result * (IgE concentration, IU/ml)	Peak IgE Concentration ** (IU/ml)	URS status
1	75.8	227.1	Positive
2	7.55	54.4	Positive
3	0.41	56.3	Positive
4	0.13	23.6	Positive
5	0.17	19.5	Positive
6	7.19	445.7	Positive
7	0.28	46.2	Positive
8	41.3	274	Positive
9	0.14	140.8	Positive
10	0.11	66.8	Positive
11	0.12	11.3	Positive
12	8.51	85.6	Negative
13	0.50	76.1	Negative
14	0.12	55.4	Negative

***** Reference range 0-0.35 IU/ml[17]; ******Reference range 0-100 IU/ml[17]

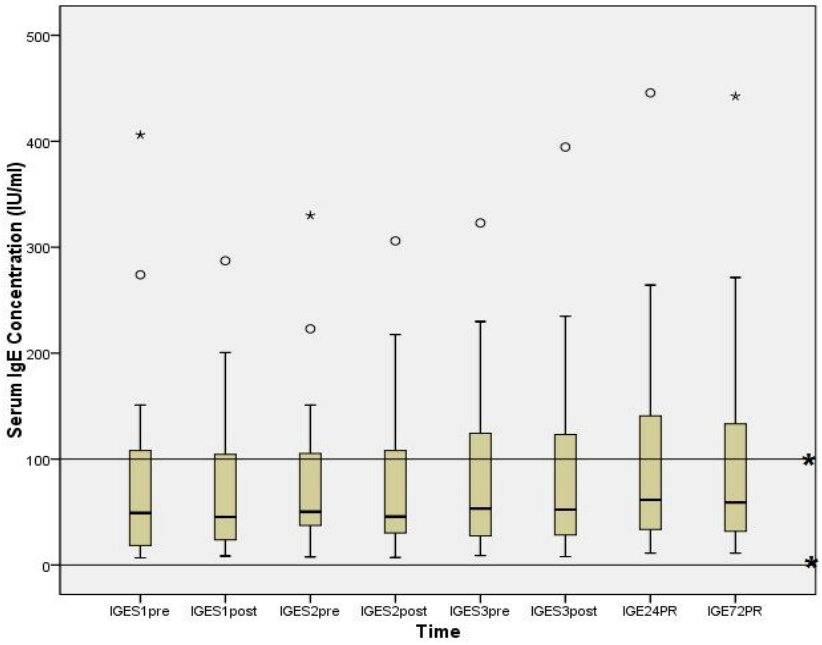

Figure 2. Median (range) of absolute serum IgE concentrations (IU/ml) of subjects at 8 time points during and after the three day, 95km trail running event. *reference range[17]

Eleven (78.6%) of the subjects presented with URS symptoms post-race (Table 3). The median (range) of total symptom scores in these symptomatic subjects is shown in Figure 3.

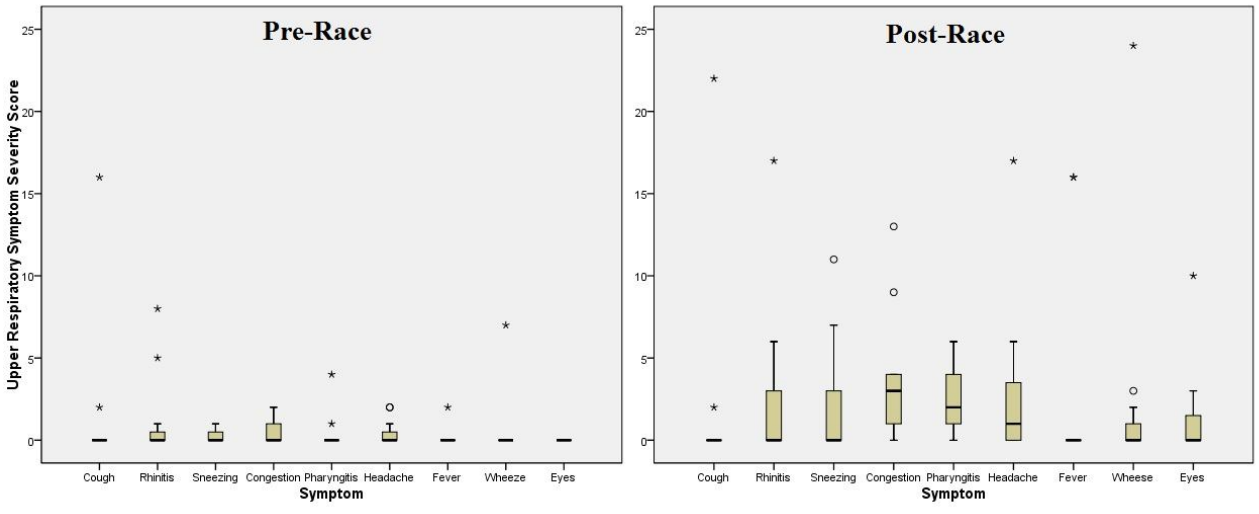

Figure 3. Median (range) upper respiratory symptom scores of the 11 URS positive subjects for the two week period before and after the event.

Individual pre- and post-race salivary IgA secretion rates (μg IgA x ml^{-1}) are presented in Table 4 along with the percentage deviation from baseline. Seven of the 14 subjects developed a greater than -40% suppression of salivary IgA secretion rate compared to baseline at some point post-race, and in two of these subjects the suppression was present at all three post-race testing periods. Of the seven cases of IgA suppression five were within the URS positive group.

Table 4: Individual salivary IgA secretion rates (μg sIgA x min^{-1}) at four stages before and after the race, as well as percentage deviations from baseline.

Case Number	$S1_{Pre}$	$S3_{Post}$	% change	24PR	% change	72PR	% change
1	268.31	59.59	**- 77.79***	98.90	**- 63.14***	102.10	**- 61.95***
2	244.13	74.57	**- 69.46***	62.06	**-74.58***	65.66	**- 73.10***
3	55.62	78.26	**40.72**	161.98	**191.25**	137.74	**147.66**
4	127.71	81.22	**- 36.40**	92.01	**- 27.95**	92.97	**- 27.20**
5	75.97	82.48	**8.56**	59.89	**- 21.17**	67.82	**- 10.74**
6	51.81	63.96	**23.45**	34.84	**- 32.75**	33.66	**35.04**
7	45.46	179.44	**294.70**	85.97	**89.09**	136.39	**200.00**
8	185.43	92.38	**- 50.18***	140.52	**- 24.22**	256.81	**38.50**
9	199.39	95.21	**- 52.25***	171.46	**- 14.00**	158.19	**- 20.66**
10	112.60	79.39	**- 29.49**	76.26	**- 32.27**	65.96	**- 41.42***
11	84.16	130.85	**55.48**	112.43	**33.60**	134.07	**59.31**
12	133.88	113.48	**- 15.24**	121.78	**- 9.04**	86.94	**- 35.07**
13	55.51	67.65	**21.87**	80.49	**44.99**	31.67	**- 42.94***
14	256.96	129.67	**- 49.54***	223.30	**- 13.10**	173.41	**- 32.51**

*sIgA value lower than -40% of their mean healthy sIgA concentration indicated a one in two chance of contracting an URTI within 3 weeks.[18]

A detailed summary of the general immunological profile and differential diagnosis of the URS positive (n=11) and URS negative (n=3) subjects is presented in Tables 5 and 6, respectively.

Table 5. Immunological profile of individual subjects presenting with URS before, during or after the event.

Case Number	Age (yrs)	Distance trained per week (km/wk)	Total finishing time (h:m)	Gender	Pre-race URS (days) *	URS During Race (days)*	Post-race URS (days)*	Phadiatop Status	IgE Elevated (range)	Absolute eosinophil concentration (range)	Absolute basophil concentration (range)	Absolute neutrophil concentration (peak)	Peak hs-CRP (mg/L)	Peak intra-stage serum cortisol conc. difference (nmol/L)	Absolute lymphocyte concentration (range)	% rise/ fall in Lymphocyte concentration 72PR	% sIgA suppression **	Differential Diagnosis (primary reason)
1	42	80	10:27	M	ST (2), C (7), RN (7)	ST (3)	BN (3), ST (6)	+	Yes (108 – 227 Peak 24PR)	NAD *** (0.03- 0.34)	NAD (0.02-0.20)	↑$S1_{Post}$ (11.2) ↑$S2_{Post}$ (12.1) ↑$S3_{Post}$ (7.7)	2.3 ($S3_{Post}$)	286	Borderline suppressed S2post (1.11)	-35%	-77.8%($S3_{Post}$), -63.1%(24PR) & -61.9% (72PR)	Reactivation of prior viral infection (lymphocytopaenia & sIgA) + Allergic Response (Phadiatop, IgE)
2	42	60	13;52	M	-	-	C (2), RN (3), S (3), BN (2), ST (2), W (3)	+	No (41 – 54)	NAD (0.07 – 0.4)	NAD (0.04- 0.08)	↑$S1_{Post}$ (11.0) ↑$S2_{Post}$ (9.6)	4.9 ($S3_{Post}$)	85	NAD (1.41-2.09)	+ 8%	-69.5%($S3_{Post}$), -74.6%(24PR) & -73.1% (72PR)	Infection (sIgA)
3	35	70	10:03	F	-	-	ST (5)	+	No (37-56)	NAD (0.06-0.44)	NAD (0.05-0.79)	↑$S1_{Post}$ (10.3) ↑$S2_{Post}$ (12.1)	7.5 ($S3_{Post}$)	123	NAD (1.66-3.13)	-23%	-	Inflammatory response (CRP)#
4	25	105	19:56	F	BN (28)	BN (3)	BN (4), ST (2)	-	No (15-23)	NAD (0.03-0.20)	NAD (0.024-0.05)	↑$S1_{Post}$ (7.6)	4.0 ($S3_{Post}$)	123	NAD (1.75-3.70)	-46%	-	Possible NAR (mild response).
5	33	12.5	12:07	F	BN (1), HA (2)	-	BN (1), HA (6)	-	No (12-19)	NAD (0.03-0.22)	NAD (0.03-0.07)	↑$S1_{Post}$ (11.4) ↑$S2_{Post}$ (10.4)	10.2 ($S3_{Post}$)	134	↓ (0.9) at S1 post (0.9-2.32)	-16%	-	Inflammatory response (CRP)#
6	44	50	17:08	F	RN(5), S (1)	BN(1)RN (1)S (1)	C (22), RN(17), S (11), BN (13), ST (2), HA (4), F (16), W (24)	+	Yes (287-445) Peak 24PR	↑24PrR (0.63) (0.11-0.63)	↑ at S2pots (0.25) ↓ at 24PR (0.00)	↑$S1_{Post}$ (9.2) ↑$S2_{Post}$ (8.4)	5.5 ($S3_{Post}$)	-88	↑ S1pre (4.42) & 24PR (4.24)	-17%	-	Infection (sIgA, Lymphocytosis) and allergic reaction (Phadiatop, IgE, eosinophilia, basophilia)
7	43	80	14:28	M	-	-	BN (1) ST (2) HA (3)	-	No (18-46)	NAD (0.05-0.26)	NAD (0.02-0.05)	↑$S1_{Post}$ (12.4) ↑$S2_{Post}$ (15.2) ↑$S3_{Post}$ (14.5)	14.9 ($S3_{Post}$)	454	Borderline ↑ (3.94) S3pre (1.48-3.94)	-34%	-	Inflammatory response(CRP)#
8	34	40	14:30	M	-	ST (2) RN (1)	RN (2), S (2), BN (4), HA (17), F (16)	+	Yes (200 – 274) Peak S1pre	↑ S1pre (0.65), S2pre(0.72) S3pre (0.73) & 24PR (0.50)	NAD (0.02-0.09)	↑$S1_{Post}$ (10.1) ↑$S2_{Post}$ (11.0) ↑$S3_{Post}$ (9.8)	116.3 (72PR)	422	↓(0.72) at 72PR (0.72-1.96)	-63%	-50% ($S3_{Post}$),	Infection (lymphocytopaenia and sIgA) and allergic reaction (Phadiatop, IgE, eosinophilia)
9	47	80	12:16	F	C (2), RN (1), S (1), BN (1), ST (4), HA (2), F (2)	-	RN (6), S (3), BN (4), ST (4), HA (1), W (2)	-	Yes (104-140) Peak 24PR	NAD (0.05-0.34)	NAD (0.03-0.08)	↑$S1_{Post}$ (10.9) ↑$S2_{Post}$ (11.7) ↑$S3_{Post}$ (9.1)	5.7 ($S3_{Post}$)	259	NAD (1.49-2.70)	-35%	-52.25% ($S3_{Post}$),	Reactivation of prior viral infection (sIgA, lymphocytopaenia) Poss. allergic component (↑IgE)
10	42	55	12:22	F	S (1), BN (1)	S (2)	RN (3), S (7), BN (9)	-	No (44.7-66)	NAD (0.07-0.39)	NAD (0.02-0.05)	-	1.7 ($S3_{Post}$)	97	NAD (1.29-2.56)	-13%	-41.4% (72PR)	Reactivation of prior viral infection
11	43	65	11:25	F	C (16), RN (8), BN (2), ST (1), HA (1)	C (1)	ST (4), HA (2)	-	No (6-11)	NAD (0.04-0.15)	NAD (0.02-0.04)	↑$S1_{Post}$ (10.8) ↑$S2_{Post}$ (9.7) ↑$S3_{Post}$ (7.6)	7.5 ($S3_{Post}$)	370	NAD (1.48-2.56)	+32%	-	Inflammatory response(CRP)#

* C = Cough; RN = Runny Nose; S sneezing; BN = Blocked nose; ST = Sore Throat; HA = Headache; F = Fever; W = Wheeze. Numbers represent the Upper Respiratory Tract Symptom Score and represent the severity of the symptom.

[18]A s-IgA value lower than -40% of their mean healthy s-IgA concentration indicated a one in two chance of contracting an URI within 3 weeks. * NAD = No abnormality detected. # serum hsCRP >5mg/L.

Table 6. Immunological profile of individual subjects not presenting with URS before, during or after the event.

Case Number	Age(yrs)	Distance trained per week (km/wk)	Total finishing time (h:m)	Gender	Pre-race URS (days) *	URS During Race (days)*	Post-race URS (days)*	Phadiatop Status	IgE Elevated (range)	Absolute eosinophil concentration (range)	Absolute basophil concentration (range)	Absolute neutrophil concentration (peak)	Peak hs-CRP (mg/L)	Peak intra-stage serum cortisol concentration difference (nmol/L)	Absolute lymphocyte concentration (range)	% rise/ fall in Lymphocyte concentration 72PR	% sIgA suppression **	Differential Diagnosis(primary reason)
12	50	70	13:35	F	S (1)	-	-	+	No 49-85)	NAD (0.04-0.32)	NAD (0.03-0.07)	$S1_{Post}$ (11.2) ↑$S2_{Post}$ (8.3)	26.9 (24PR)	299	NAD (1.5-3.67)	+44%	-	Negative: Trivial-- lasting <1 day - pre-race anxiety?
13	45	75	16:37	F	-	S (1)	HA (2)	+	No (55 – 76)	Low (0.00 – 0.1)	NAD (0.01-0.04)	↑$S1_{Post}$ (9.9) ↑$S2_{Post}$ (8.7) ↑$S3_{Post}$ (7.5)	2.5 ($S3_{Post}$)	-105	NAD (1.15-2.45)	-5%	-42% (72PR)	Negative: HA not specific to URTI; S=trivial
14	45	75	16:40	F	-	ST (1)	-	-	No (30-55)	NAD (0.03-0.11)	NAD (0.02-0.04)	↑$S1_{Post}$ (14.6) ↑$S2_{Post}$ (11.1) ↑$S3_{Post}$ (10.8)	8.2 ($S3_{Post}$)	905	Lymphocyt opaenia at S1post (0.51)	+14%	-50% (S3post)	Negative : Single symptom not lasting >1 day

* C = Cough; RN = Runny Nose; S = Sneezing; BN = Blocked nose; ST = Sore Throat; HA = Headache; F = Fever; W = Wheeze. Numbers represent the Upper Respiratory Tract Symptom Score and represent the severity of the symptom.
[18]A s-IgA value lower than -40% of their mean healthy s-IgA concentration indicated a one in two chance of contracting an URI within 3 weeks. * NAD = No abnormality detected

Of the URS positive group, six of the subjects presented with markers of the presence of infection, displaying either disturbances in absolute lymphocyte concentrations or suppression of sIgA concentrations.[18] Of these six subjects, four also presented with elevated serum IgE concentrations with concomitant post-race eosinophilia present in two and basophilia in one.

Isolated elevated serum CRP concentrations above 5 mg/L, were present in four subjects. One subject was diagnosed with possible NAR.

DISCUSSION

The incidence of URS in this study (78.6 %) is greater than that reported in most other studies investigating URS in runners.[5] This is possibly due to the nature of this long distance endurance race over three days during which athletes slept in a communal tented village, in addition to being exposed to a relatively large range of ambient temperature during the race and a forested conservancy area containing a large variety of plant allergens. Another factor to which the higher incidence of URS could be attributed was the inclusion of subjects with a prior history of allergic hypersensitivity and exclusion of subjects who reported intake of anti-histamines.

In the evaluation of the findings of the 11 URS positive cases a number of factors were taken into consideration. These include the possibility of infectious, allergic or inflammatory origin of the symptoms.

Infection?

In interpreting the lymphocyte concentrations in this study, a prolongation of the exercise-induced reduction of lymphocyte counts[5] which persisted for 72 hours following completion of the race, was regarded as clinically significant. Furthermore, depression or elevation of absolute lymphocyte count out of clinical range[17] at any stage of the race was taken as indication of reaction of the immune system to the presence of an infection.

It is well accepted that prolonged high intensity exercise is associated with decreases in both sIgA concentrations and secretion rates[5] and there is evidence to suggest that low levels of salivary sIgA or substantial transient falls in sIgA are associated with increased risk of URTI.[5,18] We used the criterion of a drop in sIgA secretion rate of more than 40% which Neville *et al.*[18] associated with a 50% risk of developing a subsequent URTI, in conjunction with a clinically significant suppression or elevation of lymphocyte count, as indicative of possible infectious causes of URS.

Six (54%) of the post-race URS positive subjects presented with findings suggestive of the presence of infectious URS which is higher than previously reported by Spence *et al.*[6] and Cox *et al.*[7] Of these six, three subjects displayed a pattern of URS during the two weeks prior to the race which is suggestive of reactivation of a viral infection. This reactivation in 21.4% of the total of 14 subjects strongly supports the findings of Reid *et al.*[8] who documented reactivation of the Epstein Barr virus in 19.5% of subjects.

Allergy?

The incidence of allergic diseases, which can closely mimic URTI symptoms[19] is increasing[13,20-22] with the cited rates of allergic rhinitis in athletes currently being higher than in the normal population.[22] The Phadiatop® assay which measures specific serum IgE concentration in response to 23 possible inhaled allergens,[14] was positive in 50 % of the sample and confirms previous findings of Schwellnus *et al.* of the large percentage incidence of pre-race allergic hypersensitivity.[23]

Furthermore, participants using anti-histamines (n=2) were excluded from the study due to the effect of this medication on URS[21] in order to increase the validity of our differential diagnoses. Of interest is the low dependence on anti-histamines in our group (n=22) confirming the findings of Randolf *et al.*[24] in which only 0.5% were using anti-histamine medication.

Although the eosinophilia which is typical of atopic asthma and allergic rhinitis,[25,26] is characteristically most detectable in local tissue and mucosal secretions, mild serum eosinophilia is also commonly induced by allergic reactions.[26] In this study two of the subjects developed

clinically significant eosinophilia. Both of these subjects presented with simultaneous elevations in serum IgE, in addition to being Phadiatop® positive and symptomatic.

The contribution of basophils to the acute phase allergic response by amplifying the production of IgE is well recognised.[26] In this study, one subject displayed clinically significant basophilia[17] along with eosinophilia, raised serum IgE concentrations[17] and URS, suggestive of an allergic hypersensitivity reaction.

While the protective role of serum IgE against reactive airway disease, is well documented in susceptible individuals, significantly elevated serum IgE concentrations are also indicative of the hypersensitivity reactions occurring during atopic diseases such as allergic rhinitis.[26] Therefore eosinophilia or basophilia detected in conjunction with elevated circulating IgE concentration and self-reported URS was taken to represent the activation of an allergic process in this study.

Of greatest interest was the finding that markers of an isolated allergic response were not present in these 14 case studies. In all four cases that presented with positive markers of an allergic reaction, it was associated with markers of *de novo* infection or possible reactivation of prior viral infection.

Inflammation?

In this study elevations of serum hs-CRP concentrations above 5mg/L were used to detect the presence of an acute phase reaction. As this may however have been due to a broad range of acute or chronic inflammatory responses to the presence of infection or muscular inflammation**,** the overall immunological profjile of each individual was considered before making a differential diagnosis. Therefore only in the absence of clinically significant indicators of infection, were isolated elevations in CRP concentrations regarded as indicative of an inflammatory response.

An isolated systemic acute phase response which may have triggered an inflammatory response within the respiratory tract and explain the development of non-infectious sore throats and

rhinitis,[3,5,10] was evident in four out of the 11 (45.4%) of the sample presenting with URS in the absence of systemic markers of concomitant infection or allergic reaction.

Firstly, we confirm previous findings that raised hs-CRP is not linked to allergic responses.[27] The hs-CRP concentrations did also not increase to beyond the recommended range for the presence of mild inflammation[28] in these four subjects and further as yet unpublished data from our laboratory,[29] reflect the absence of evidence of substantial skeletal muscle damage (serum creatine kinase concentrations > 2000U/L). It would nevertheless have been advisable to have confirmed this diagnosis by examining the sputum for markers of local inflammation in the respiratory tract.[3] This would be recommended as a consideration for future studies.

Non-allergic rhinitis? (NAR)

Although NAR mimics allergic rhinitis, it presents with normal serum IgE levels and a negative Phadiatop® test for atopy[30,31] and the prevalence in the adult population is believed to be at least 25%.[31] The exact cause is unknown, but the underlying inflammatory mechanisms that have been postulated in athletes presenting with conditions such as "runners nose,"[13] have been related to repeated dehydration injury, extreme ambient temperatures, irritant pollutant and allergen exposure and cortisol and catecholamine fluctuations.[31] In this study, there appeared to be evidence of this condition in one of the 14 subjects surveyed in whom the elevation in CRP was so slight that NAR could possibly be suggested.

Conclusion

Taken together, the findings of these 14 case studies indicate the absence of allergic responsiveness alone in the aetiology of URS in these trail runners. Evidence of allergic responsiveness was present in four subjects, but in each case this was linked to systemic indicators of the presence of a concurrent infection, rather than inflammation.

Although the actual cause and effect relationship between allergic responsiveness and a concomitant infectious response has not yet been confirmed, the findings of this study appear to suggest that anti-histamine or other anti-allergy medication alone will not suffice in the treatment of these exercise-induced symptoms in the respiratory tract.

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CHAPTER 4 – SCIENTIFIC PUBLICATION 2

As the analysis conducted in chapter 3 revealed that not all of the athletes with elevated serum IgE concentrations were Phadiatop positive, it was decided to conduct a retrospective analysis of the validity of Phadiatop status in predicting pre-disposition to allergy-associated RTS. As serum IgE concentrations are not affected by the intake of anti-histamines, subjects using these medications were included in this analysis which was submitted and accepted for publication in the S. A. Medical Journal (Appendix A).

This is the first analysis of the validity of Phadiatop testing when applied to trail runners participating in a country setting in South Africa.

Phadiatop® testing in assessing pre-disposition to Respiratory Tract Symptoms of allergic origin in athletes?

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ABSTRACT

OBJECTIVES:

To validate the use of the Phadiatop® test as a predictor of allergy-associated Respiratory Tract Symptoms (RTS) in trail runners.

METHODS:

The incidence of self- reported RTS was documented in 16 runners for 31 days and related to the Phadiatop® status and circulating markers of allergic responses at 8 time points before, during and after a three-day 95 km trail run.

OUTCOME MEASURES:

Incidence of RTS, pre-race Phadiatop® status, changes in concentrations of serum IgE (sIgE), differential leukocyte counts.

RESULTS:

Twelve (75%) athletes presented with post-race RTS and seven (58%) of these were Phadiatop® positive. In only four (57%) of the symptomatic subjects who had positive Phadiatop® status, was the RTS accompanied by peak sIgE concentration > 100 IU/ml. There was no significant difference between the eosinophil and basophil concentrations of the positive and negative groups ($p>0.05$). One of the Phadiatop® negative subjects presented with RTS as well as peak sIgE concentration > 100 IU/ml.

CONCLUSION:

The Phadiatop® assay does not accurately predict the development of post-exercise RTS of allergic origin in trail runners.

INTRODUCTION

Of concern since the early 1980's has been the high incidence of upper respiratory tract infections in athletes during periods of intensive exercise training and exhaustive endurance events.[1,2] This has resulted in interrupted training schedules and impaired performance in competitive events. For many years exercise immunologists have sought methods of identifying the cause of these symptoms which have now been extended to the lower respiratory tract.[3]

It has been shown that 30%-40% of post exercise Respiratory Tract Symptom (RTS) cases are the result of infection, another 30% resulting from inflammatory causes and the final 30% resulting from unknown causes.[2,4-6] Numerous theories have been proposed in the development of non-infective post-exercise RTS, including the development of hyper reactive airways,[7] a run-away inflammatory response,[8] a reactivation of latent viral infection [6] and allergic reaction.[3,9]

Athletes have been documented to experience higher rates of allergic diseases than the general population[10] and that the cited incidence of allergy among Olympic athletes is steadily increasing.[11] As exercise-induced symptoms of infection of the respiratory tract can closely mimic those of an allergic reaction[3] and exercise is known to induce a T_H2 dominant immunological shift[2], it may up-regulate an allergic response in those already sensitized,[9] and increased exposure of athletes to irritants and allergens[3,11] may contribute towards this.

Although the skin prick test (SPT), is generally accepted as the standard method for detecting IgE related allergic sensitization[12] its limitations include lower response in the elderly, difficulty in grading the response in darkly pigmented persons, contraindication for the pregnant, the quality and selection of allergens and potential risk of anaphylaxis.[13]

Specific IgE antibody testing is accepted as an alternative to the SPT[12] and combination tests such as the Phadiatop® assay (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) simultaneously test for IgE to a mixture of allergens causing common inhalant allergies. Allergens included in the Phadiatop® assay are Artemisia, dust mites (D. pteronyssinus and D. fariane), mixed moulds (Penicillium, Cladosporium, Aspergillus and Alternaria), pet dander (cat

and dog), mixed grasses (Parietaria, Lolium, Phleum and Cynodon), and mixed trees (Acer, Betula, Olea, Salix, Pinus, Ulmus, Quercus, Eucalyptus, Acaciaand Malaleuca).[14] This assay has been found to offer satisfactory accuracy for diagnosing of IgE allergic sensitization in the general population with a sensitivity of 70.8% and a specificity of 90.7% compared to the SPT.[12]

The primary objective of this study was therefore to investigate the validity of the Phadiatop® test as a predictor of allergy associated RTS in athletes competing in a 3 day, 95km, trail run. The secondary objective was to document the incidence of RTS pre-, during and post-event and relate these to the concentrations of serum IgE, leukocyte parameters and Phadiatop® status of the athletes throughout and after the event.

METHODS

This longitudinal study was part of a larger study examining physiological responses during the Three Cranes (a 95km, three-day trail run) in Karkloof, KwaZulu-Natal on 25-27 February 2011. Local institutional ethical approval was obtained and a sample of 22 volunteers signed informed consent forms.

After routine baseline testing on the afternoon before the race, venous blood samples were collected at a total of eight time-points, before and after each day's stage ($S1_{pre}$, $S1_{post}$, $S2_{pre}$, $S2_{post}$, $S3_{pre}$ and $S3_{post}$), 24 hours post race (24PR) and 72 hours post race (72PR). RTS data was collected over a 31 day period, from 14 days prior to the race until the 14^{th} day after the race. Of the 22 subjects, 16 completed the race and complied with all the study requirements. Two subjects were excluded as a result of failure to complete the race and a further four did not complete post race testing.

Athletes were asked to record the daily incidence and severity of RTS pre, during and post-race using a graded 1-3 point scoring system. Symptoms monitored included cough, runny nose, sneezing, blocked nose, sore throat, headache, fever, tight chest and itchy eyes. A total respiratory tract symptom index score was determined using the sum of severity scores mentioned above and the length of time that the symptom presented.

To determine which subjects qualified for post-exercise RTS, any subjects presenting with a single RTS lasting < 2 days or any non-specific symptom (e.g. headache, itchy eyes) not accompanied by a RTS lasting > 1 day, were excluded. A peak post-stage or race serum IgE (sIgE) concentration below the clinically significant range (100IU/ml) excluded the possibility of the RTS being of allergic origin.

Full blood counts, pre-race Phadiatop® status and sIgE concentrations were determined by a local pathology laboratory (Ampath Laboratories, Howick) using an automated UniCAP system. In the Phadiatop® assay, concentrations higher than 0.35 IU/ml represented a positive response irrespective of range.[14]

Exercise-induced changes in plasma volume (PV) over this three day event were determined from pre- and post-exercise haematocrit and haemoglobin concentrations.[15] Post exercise sIgE and concentration dependant leukocyte counts were adjusted for percentage exercise-induced change in PV.

After confirmation of the absence of normality of the data, they were logarithmically transformed. A Generalized Linear Model was applied to the median (range) sIgE and differential leukocyte concentration data from multiple subjects over multiple time points and between Phadiatop® positive and negative groups. The Mann-Whitney U test was used to compare the RTS data pre- and post-race and between Phadiatop® positive and negative groups. Data are presented as the median (interquartile range, IQR) in box and whisker plots in Figures 1 and 2. Significance was set at $p=0.05$.

RESULTS

Of the 16 subjects (12 female, 4 male; 25-50 years), nine were Phadiatop® positive and seven, Phadiatop® negative. The median and range of BMI (23.8, 18-7 - 27.7 vs. 20.9, 19.7 - 25.3) and % body fat (20.9, 15.7 - 29.3 vs. 23.7, 17.2 - 30.6) did not differ significantly between Phadiatop® positive and negative groups. Baseline testing of vital signs were within the normal

range and subjects did not present with evidence of any medical condition that could place their health at risk. The results of the Phadiatop® assay and peak sIgE concentrations are presented in Table 1.

Table 1. Phadiatop® assay results and peak race-induced sIgE concentrations of the subjects (n=16).

Phadiatop® Positive (n=9)			**Phadiatop® Negative (n=7)**		
Subject Number	**Phadiatop® Result* (sIgE concentration, IU/ml)**	**Peak sIgE concentration (IU/ml)** (time-point)**	**Subject Number**	**Phadiatop® Result* (sIgE concentration, IU/ml)**	**Peak sIgE concentration (IU/ml)** (time-point)**
1	8.51	85.64 (24PR)	**5**	0.13	23.64(24PR)
2	75.80	227.14(24PR)	**6**	0.17	19.46(72PR)
3	7.55	54.38(24PR)	**10**	0.12	55.44(24PR)
4	0.41	56.32(24PR)	**12**	0.28	33.58(24PR)
7	7.19	445.74(24PR)	**14**	0.12	11.30(24PR)
8	3.42	240.48(72PR)	**15**	0.14	140.82(24PR)
9	0.50	76.13(24PR)	**16**	0.11	66.79(24PR)
11	9.49	63.14(24PR)			
13	41.30	274.0($S1_{pre}$)			
Median	**7.55#**	**85.64#**	**Median**	**0.13**	**33.58**

* SIgE Reference range: Phadiatop® 0.00-0.35IU/ml

** Reference range: Total SIgE 0.00-100 IU/ml

# $p < 0.001$ vs. Phadiatop® negative group, Generalised Linear Model

Twelve subjects (75%) met the criteria for post race RTS. Of these, seven (58%) were Phadiatop® positive and five (42%) were Phadiatop® negative. The median (IQR) pre-and post-race RTS index scores of the Phadiatop® groups did not differ significantly ($p>0.05$, Figure 1).

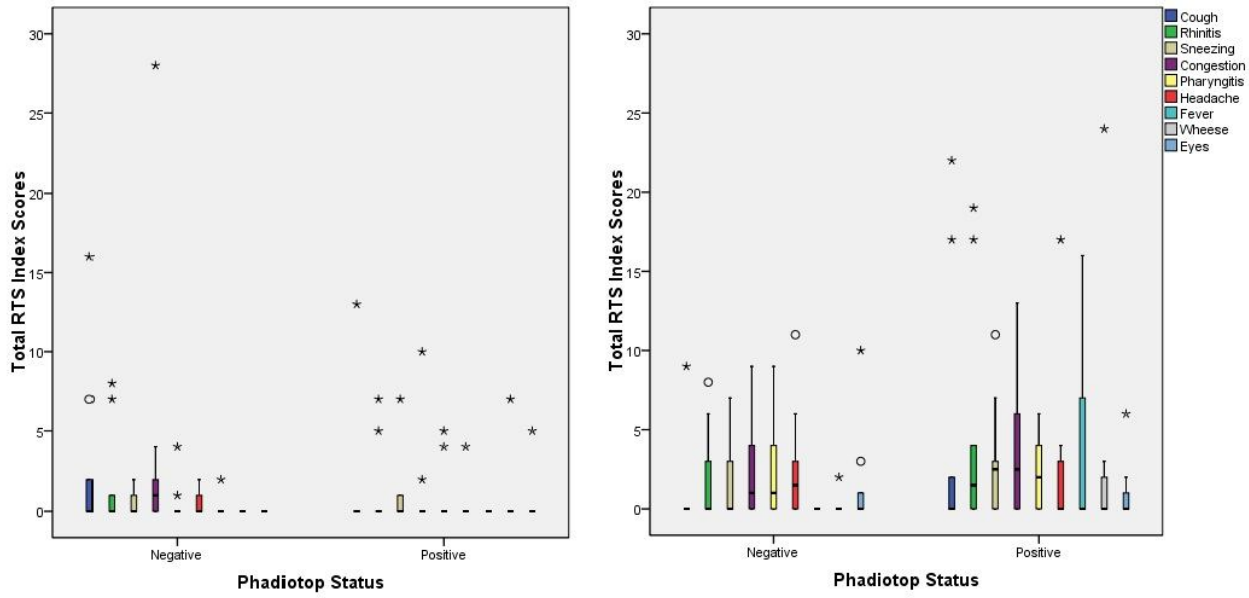

Figure 1. Median (IQR) pre-race (left) and post-race (right) total RTS index scores of Phadiatop® positive* (n=9) and negative (n=7) groups.

*sIgE concentration >0.35IU/ml in Phadiatop® assay.

The median (IQR) sIgE concentrations of the subjects (adjusted for PV) are presented in Figure 2. There was a non-significant ($p=0.37$) rise in the sIgE concentrations of the entire group over the course of testing, with highest concentrations being recorded in 75% of subjects at the 24PR time period. There was a highly significant ($p<0.001$) difference between the sIgE concentrations of the Phadiatop® positive and negative groups.

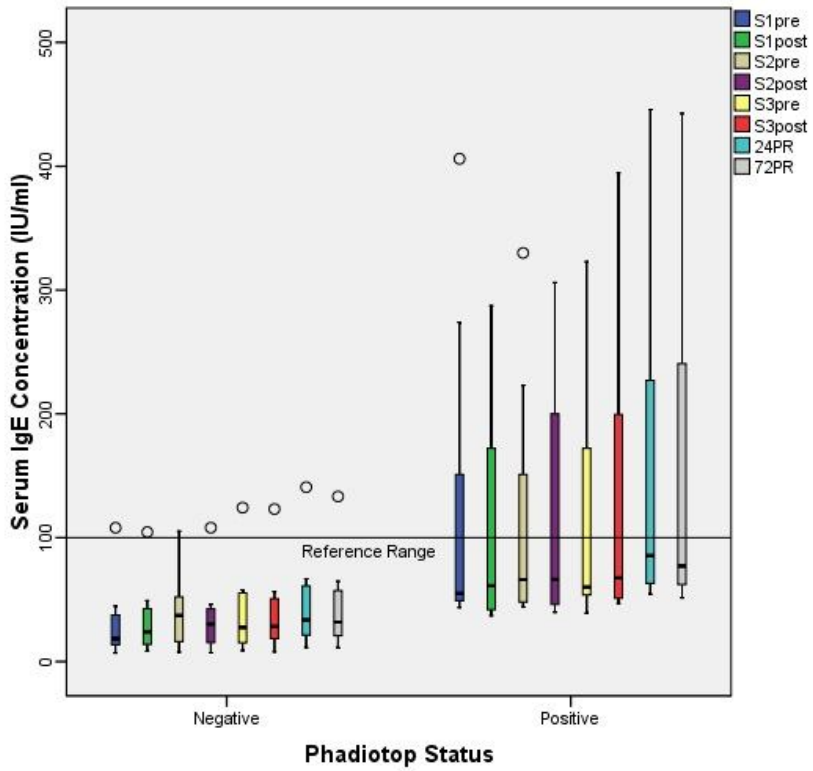

Figure 2. Median (IQR) of absolute sIgE† concentrations* of Phadiatop® positive and negative groups, at 8 stages during and after a 3 day, 95km trail run.

†reference range: 0-100IU/ml

*adjusted for plasma volume

Concentrations of sIgE reached clinical significance (peak sIgE concentration > 100 IU/ml) in five (42%) of the 12 RTS positive subjects (four Phadiatop® and RTS positive subjects and one Phadiatop® negative and RTS positive subject).

There was no significant exercise-induced elevation in PV adjusted concentrations of either basophils or eosinophils over time ($p>0.05$) and the difference between Phadiatop® positive and negative groups in terms of eosinophil or basophil response to three days of exercise was not significant ($p>0.05$).

DISCUSSION

The incidence of post exercise RTS in this study (n=12, 75%) was higher than most other studies investigating runners have reported.[1-3] This was possibly due to inclusion of subjects with prior history of allergy, as in most previous studies allergy was seen as a confounding factor for the determination of URTI incidence and excluded.

Interestingly, in this field trial subjects with systemic evidence of RTS associated with an allergic reaction accounted for 42% (n=5) of the 12 cases with post-exercise RTS. However as seen by the lack of significance between post race RTS incidence in Phadiatop® positive and negative groups (Fig 1), the incidence of post race RTS symptoms was not defined by the Phadiatop® test.

The primary finding of this study was however that of the seven Phadiatop® positive subjects who developed post-race RTS, only four (58%) displayed clinically elevated sIgE concentrations above the cut off point for allergy (sIgE > 100IU/ml). In addition, the mildly elevated eosinophil concentrations often seen in allergic responses[13] were not evident in the Phadiatop positive group ($P>0.05$).

Although specific sIgE antibody testing (or SPT) provides evidence of sensitization to an allergen, an allergic disease response only develops once the individual is exposed to that particular allergen. Therefore although the Phadiatop® test may provide satisfactory accuracy in identifying predisposition to allergic responsiveness to airborne allergens, on its own, it may not predict the development of allergic disease in trail runners. Due to the fixed selection of allergens it is theoretically possible to miss subjects who are sensitized to less common inhalant allergens (such as local flora or fauna), as was the case in one of our subjects.

The predictive validity of the Phadiatop® assay for the incidence of race-induced RTS of allergic origin in trail runners must therefore be questioned.

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CHAPTER 5 – CONCLUSIONS

The present study was designed to examine the role that allergic predisposition or reaction plays in the development of post exercise RTS. Having examined the results of the studies it is now possible to return to the hypotheses stated at the beginning of the study.

1) Firstly, this work supported the hypothesized increase in RTS within the post race period. Both analyses showed an incidence of post-exercise RTS equal to or greater than 75 %, which is higher than previous studies involving runners. This is possibly due to the inclusion of allergic subjects within the test group.

2) Secondly, it was also hypothesized that the effect of the three-day trail run on systemic markers of immune system activation would be cumulative. The race did result in a cumulative effect on hs-CRP concentrations and an exercise effect was noted in total leukocyte, neutrophil and lymphocyte concentrations (as is to be expected after exhaustive exercise), resulting in lasting perturbations. The effect on other differential cell count parameters however, was not significant and concentrations had returned to pre-race concentrations within 72 hours of the final stage. The three-day race did not have a strictly cumulative effect on sIgA concentrations, with only seven subjects showing suppression and only two of those being cumulative. No cumulative effect was noted for markers of allergy, although mean exercise-induced sIgE concentrations rose throughout the testing period, the effect was not statistically significant (unlike the findings of Aldred *et al*. 2010) and no cumulative effect was noted in eosinophil or basophil concentrations.

3) Thirdly, the fact that the incidence of RTS was not related to any single systemic or salivary marker of immune function was confirmed. As a series of 14 case studies was undertaken of the general immune profile and RTS status of trail- running subjects, it is possible to confidently support the above hypothesis. Subjects who were positive for post race RTS, presented with a variety of disturbances in immune parameters with no one marker displaying a dominant or significant relationship. Athletes with RTS

presented with a variety of allergic, infectious and inflammatory markers reflecting a more multi-faceted aetiology to their symptoms.

4) Fourthly, it was found that Phadiatop® positive subjects (n=9) did not present with raised markers of allergic activation, as only 44% (n=4) had clinically elevated sIgE concentrations at any point during the event.

5) Finally, it was demonstrated that the Phadiatop® Assay does not accurately predict the development of post exercise RTS in trail-runners.

Although this study did not demonstrate an exclusive connection between allergy (either reaction or predisposition towards) alone and the development of post exercise RTS, it did show that a large percentage of symptomatic subjects display signs of allergic reaction (36/42%). Taken together, these results suggest that, whilst new infections, reactivation of previous infections and inflammation all appear to contribute to the development of post exercise respiratory symptoms, allergy cannot be discounted as a contributory factor. Based on this it is recommended that runners presenting to their physician with post exercise RTS, or a history of recurring RTS, be examined comprehensively for infection, chronic viruses, other systemic inflammatory disease and allergic reaction.

One of the most significant findings to emerge from this study is that allergic reaction, or clinically elevated sIgE concentrations were only evident in subjects with concomitant immune perturbations associated with increased risk of infection. This raises the question whether having clinically elevated sIgE concentrations increases susceptibility to infectious post exercise RTS? This potential link between allergic reaction and infectious RTS requires further study.

Another major finding was that the Phadiatop® assay cannot be used to screen for an increased susceptibility toward RTS. It is also concerning that this assay cannot be adjusted to account for local flora and fauna and that it does not cover food allergens. Therefore it is recommended that skin prick testing for a battery of routine allergens be used to diagnose atopic predisposition and total sIgE concentrations be used to assess tendency toward allergic reaction. Furthermore, recreational athletes appear to take little allergy medication (9% antihistamines in this study and

0.5% anti-histamine usage by recreational runners before a race observed by Randolph *et al.*, (2006)). It would therefore be interesting to determine whether medications that lower sIgE concentrations or block allergic reactions may reduce the incidence of infectious RTS.

The findings in this study are subject to a number of limitations. Firstly, the project used a convenience sample that may not have been equally representative of gender and racial demographics. Secondly, the scope of this Masters (by course-work) project and concomitant financial considerations necessitated a limitation of the number of immunological tests that were performed. Thirdly, infection or lack thereof was not confirmed through physician examination, culture of swabs or x-ray, blood test results and self reported data were relied upon. Lastly, the extensive level of commitment required from each subject, who was requested to fully comply with all requirements of this work over a 31-day period, limited the number of athletes who were prepared to make themselves available to participate in the study as well as the number of athletes who were able to fully comply.

It is therefore recommended that future studies in this area carefully explore strategies of increasing the number of subjects. Additionally a detailed physical examination of subjects presenting with post-race RTS, with the aim of detecting and identifying pathogens, is recommended. This would aid in differentiating between allergic reaction and infectious RTS and help to define the relationship between these two factors. Furthermore, exploration of adaptations to the current Phadiatop® assay which would increase its predictive validity for trail runners in South Africa is another important future research consideration.

Despite its limitations, this Masters (by course-work) project has produced two preliminary novel findings that have gone some way towards developing the understanding of the aetiology of allergic respiratory symptoms in trail runners and the direction of future research in this area.

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APPENDIX A

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14 February 2012

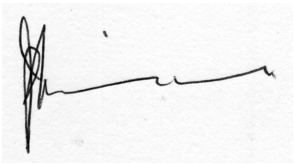
Dr Anton de Waard
Sports Medicine
Division of Human Physiology
School of Laboratory Science and Medical Sciences
College of Health Sciences
University of KwaZulu-Natal

Dear Anton

Re: **Phadiatop® testing in assessing pre-disposition to respiratory tract symptoms of allergic origin in athletes?**

This is to confirm that your paper has been accepted for publication in the South African Medical Journal.

Kind regards
JP



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APPENDIX B

DEPARTMENT OF PHYSIOLOGY

COLLEGE OF HEALTH SCIENCES

Physiological assessment of participants in a three-day trail run event: markers of muscle damage, hydration and immune status.

SUBJECT CONSENT FORM

I, …….. hereby agree to participate in a research study to be performed by Dr. Rentia Denissen, Dr Anton de Waard, Mr. Navin Runjit Singh and Professor Edith Peters-Futre in the Department of Human Physiology in the College of Health Sciences of the University of KwaZulu-Natal. I have been informed about the study by the principal investigator, Professor Peters-Futre.

I understand that the basic procedures to be carried out are to include:

1. Completion of URTI questionnaire for 14 days prior to the race
Assessment of height, mass and percentage body fat, heart rate, blood pressure and peak flow prior to registration on 24 February
2. Completing two questionnaires as well as providing a small vial of blood (2ml) to perform an allergy test at the pre-race briefing
2. Completion of a brief questionnaire, giving a urine and saliva sample and the taking of three vials of blood (a total of no more than 14ml of blood), twice daily (before and after each stage of the three-day event)
3. Completion of a post-stage questionnaire, giving a urine sample and three vials of blood 24, 48 and 72 hrs after the race finishes
4. Accurately measuring all fluid consumed and urine voided throughout the duration of the race, and recording the incidence of upper respiratory tract infection symptoms during the 2-week post-race period
5. Completion of a URTI questionnaire for 14 days following the race

The details of these procedures have been explained to me in full. I am aware that a certain level of discomfort may occur when the blood is taken and that this procedure may be accompanied by certain medical risks including infection and inflammation of the vein.

I understand that the study forms part of the Masters degrees of Drs Rentia Denissen and Anton de Waard and that the results may be published.

I understand that participation is entirely voluntary and that I may withdraw from the study at any time.

I may contact the principal investigator of the project, Professor Peters Futre at 073- 7597974 at any time if I have questions about the research or if I are injured as a result of the research.

_____ _____

Signature of Participant Date

_____ _____

Signature of Witness Date (Where applicable)

APPENDIX C

PRE RACE	SELF REPORTED URTI SYMPTOMS	**Severity Rating**: 0 = Nothing; 1 = Mild 2 = Moderate; 3 = Severe

Days	Cough	Runny Nose	Sneezing	Blocked nose	Sore throat	Headache	Fever	Wheeze / tight chest	Watery / Itchy Eyes
11 Feb									
12 Feb									
13 Feb									
14 Feb									
15 Feb									
16 Feb									
17 Feb									
18 Feb									
19 Feb									
20 Feb									
21 Feb									
22 Feb									
23 Feb									
24 Feb									

POST RACE	SELF REPORTED URTI SYMPTOMS	**Severity Rating**: 0 = Nothing; 1 = Mild 2 = Moderate; 3 = Severe

Days	Cough	Runny Nose	Sneezing	Blocked nose	Sore throat	Headache	Fever	Wheeze / tight chest	Watery / Itchy Eyes
25 Feb									
26 Feb									
27 Feb									
28 Feb									
1 March									
2 March									
3 March									
4 March									
5 March									
6 March									
7 March									
8 March									
9 March									
10 March									
11 March									
12 March									
13 March									