Ana Teixeira & Luis Rama
Editors

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July 9, 2010 - PROGRAM

9.00 • **Cerimónia de Abertura**
Prof. Dr José Pedro Ferreira **(Dean of Faculty of Sport Sciences and Physical Activity of Coimbra University**
Drª Luisa Pais **(Director of Center of Histocompatibility of Coimbra)**

9.15 •Ricardo Costa **The Potential Role of Exercise and Diet in the Management of Obesity-Induced LowLevel Chronic Inflammation** *Coventry University*, UK

10.00 • Ana Teixeira & Luís Rama **Immune changes potentially associated with upper respiratory tract infections in swimmers during a winter training season** *University of Coimbra, Portugal*

10.45 • **Coffee-break**

11.00 • Neil Walsh (Ricardo Costa). **Nutritional countermeasures for exercise-induced immune impairment** *Bangor University, UK*

12.15 • Maria Paula Mota **The Influence of Aerobic Fitness on Age-related Lymphocyte DNA Damage in Humans: some possible underlying mechanisms** *University of Trás-os-Montes e Alto Douro, Portugal*

13.00 • **Lunch**

14.15 • Margarida Figueiredo Braga **Affective state and resilience to stressful situations: psychosocial andbiological correlates** *University of Porto, Portugal*

15.45 • Cristina Bento **Immune system in elite swimmers and combat sports athletes** *Technical University of Lisbon, Portugal*

16.15 • **Coffee-break**

16.45 • Artur Paiva **Phenotypic and Functional characterization of peripheral blood dendritic cells** *Center of Histocompatibility, Coimbra, Portugal*

17.15 • Nicholas West & Maree Gleeson **Cytokine responses to exercise and gene polymorphisms associated with risk ofupper respiratory infections** *Newcastle University, Australia*

Índex

PREFACE

Immunity in sport has been receiving attention from researchers in recent years, which seems to be related to the high demands of sport preparation. Theinfluence of training load on immunity status in different environments of sporting participation together with the relationship between training load and the immune responses has been on the focus of a large body of research. Often literature reports that high level competitive athletes seem to be susceptible to recurrent infections, namely at the upper respiratory and gastrointestinal tract. Most research has focused on the acute response, but athletes that train day by day 3 to 4 hours a day are subjected to large amounts of stress that could affect their immune function and health. This could impair their training and competitive condition.

The main objective of this symposium was to join the Portuguese researchers interested in sport and immunology, that often work many times alone, and with the contribution of researchers from other countries, look at the sate of art of sport immunology research in our country. Connecting, for a common future!

Ricardo Costa focus on sustained military training and routine operations, adventure and exploration activities, ultra-endurance competitions, and traveling across time zones for sports competitions that are commonly associated with a variety of immune modulating physiological stressors (e.g. prolonged strenuous exercise, sleep-deprivation, nutrient deprivation, exposure to environmental extremes). The accompanying perturbations to host defenses may have significant clinical implications; such as, increasing the risk of illness, infection, dampening effective wound healing and increasing susceptibility to sepsis following injury in the field. Especially when activities are performed in unknown environments, and/or extensive contact with the surrounding population exists after stress stimuli, whereby an increased number of unrecognizable foreign opportunistic pathogens are present.

Ana Teixeira, Luis Rama and colleagues looked to the seasonal variations in training loads and to the influence on the immune status of athletes undergoing systematic and heavy training programs. Changes in lymphocyte subsets after

exercise have been well documented, but fewer studies have looked at the responses of distinct subpopulations that may reflect functional changes during a training season. The aim of their presentation is to see how training load might influence the proportion and phenotypic features of circulating leukocytes, B, T and NK lymphocytes and T cells subsets in peripheral blood (PB) of high competitive levels athletes and how it could affect susceptibility to disease

Because events of short durationwith programs of energy restriction lasting only 1-2 days decrease immune function (e.g. an athlete making weight for competition), Neil Walsh focus on nutrition to provided the athlete a well-balanced diet that meets energy needs and the intake of macronutrients and micronutrients that will be sufficient to maintain immune health. This talk reinforces the idea that carbohydrate ingestion during exercise or shortly after exercise can attenuate the immune impairment. A high carbohydrate diet will not only optimize athletic performance but may also decrease the immunosuppressive effect of stress hormones. Athletes on energy restricted diets should consider a low-dose vitamin supplement.

Maria Paula Mota group center their talk on the age related increase of accumulated products of DNA damage, causing progressive enhanced susceptibility to disease and risk of death. Moreover, the age-related increased concentration of DNA-oxidized bases clearly connects oxidative stress with the aging process and supports the concept of the existence of DNA mutation accumulation throughout life in organs and systems, including the immune system. Although different origins and mechanisms have been proposed to explain the occurrence of DNA mutations,this authors highlight the possibility that mitochondria play an important role in age-related immunodeficiency. Indeed, age-related oxidative damage to mitochondria results in a progressive reduction in mitochondrial bioenergetic capacity, leading to cellular energy deficits that compromise overall cellular functionality and to an increase in ROS formation and oxidative stress which damage cell macromolecules such as DNA.

Margarida Figueiredo Braga presents data that support the idea that sustained stress, major stressful Life Events, social stress and subjectively perceived daily stress can significantly impact psychological and physical well being, It is shown that a wide range of short and long-term biological effects including immunological, neuroendocrine and neurotransmitter alterations can result from exposure to stressful situations, which also can contribute to precipitate or exacerbate psychological disturbances.

Evidence has been shown that people with more positive affect overall adapt more readily during times of stress. Furthermore, the dynamic quality of the relationship between positive and negative affect points to a moderating effect of affective state in psychological and biologic individual stress response.

Correlations of Affective State with psychosocial and immunological markers point to a link between emotional state and reduced risk of physical illness, less damaging stress responses and a resilience to psychological suffering, stimulating the search for biologic and behavior mediators.

Cristina Monteiro Bento highlights that exercise, both high-intensity and prolonged, is a stress to the body that is proportional to the intensity and duration of the exercise, relative to the maximal capacity of the athlete. Exercise stress leads to a proportional increase in stress hormones, such as cortisol and catecholamines and concomitant changes in several aspects of immunity including neutrophilia, lymphopenia, decreases in granulocyte oxidative burst, NK cell activity, lymphocyte proliferation, and production of cytokines in response to mitogens, increases in granulocyte and monocyte phagocytosis, and in blood pro and anti-inflammatory cytokines, and nasal and salivary immunoglobulin A (IgA) levels. Adrenaline and noradrenaline mediate the acute effects of exercise on lymphocytes, whereas cortisol mediate neutrophils. Physical exercise is also associated with accelerated reactive oxygen species (ROS) generation. As a consequence conditions may be established where ROS production may overwhelm the antioxidant defences and consequently induce damage to macromolecules that may either compromise or stimulate the immune system.

The importance of the functions that dendritic cells, neutrophils and monocytes play in protecting against infections is well understood. However few studies have looked at the effect of a training season on these cells. We found no studies on dendritic cells and sport and physical activity. The presentation of Artur Paiva and colleagues aims to examine the effects of intensive and prolonged periods of training on various components of the innate immune system exploring the influence of a normal training program on the functional features of peripheral blood dendritic cells, monocytes and neutrophils in high competitive level athletes engaged in an endurance sport.

Nicholas West and Maree Gleeson support their presentation on the concept that Upper respiratory illness (URI) and the associated symptoms is a significant contributor to reducedperformance in high performance athletes, however the aetiology of these symptoms is still uncertain. Post-exercise disturbances in pro- and anti-inflammatory markers differ in illness-prone and healthy athletes and may contribute to the symptoms experienced by some athletes. Single nucleotide changes (polymorphisms) in cytokine genes controlling immune and inflammatory responses and alterations in the associated gene expression may influence the risk for recurrent episodes of URS in athletes. The aim of the study they presentedwas to compare the frequency of cytokine gene polymorphisms between healthy high-performance athletes and athletes prone to frequent URS.

The Influence of Sleep-Deprivation, Cold-Exposure, Exercise Stress and Nutritional Intervention on Leukocyte Trafficking, Bacterially-Stimulated Neutrophil Degranulation and Saliva Secretory Iga Responses.

Ricardo Costa

Coventry University, UK

Introduction

Sustained military training and routine operations, adventure and exploration activities, ultra-endurance competitions, and travelling across time zones for sports competitions are commonly associated with a variety of immune modulating physiological stressors (e.g. prolonged strenuous exercise, sleep-deprivation, nutrient deprivation, exposure to environmental extremes). The accompanying perturbations to host defences may have significant clinical implications; such as, increasing the risk of illness, infection, dampening effective wound healing and increasing susceptibility to sepsis following injury in the field. Especially when activities are performed in unknown environments, and/or extensive contact with the surrounding population exists after stress stimuli, whereby an increased number of unrecognisable foreign opportunistic pathogens are present (Martinez-Lopez et al., 1993; Shephard et al., 1998).

Substantial evidence suggests that individual physiological stressors may play a key role in causing impaired host defences (Nieman, 1994; Dinges et al., 1995; Shephard et al., 1998; Walsh & Whitham, 2006). This appear to be primarily induced by stimulating neuroendocrine mechanisms, such as the Hypothalamic-Pituitary Adrenal (HPA) and Sympatheticoadrenal-Medullary (SAM) axes that modulates immune responses through neurotransmitter, neuropeptide and neurohormonal receptor sites on lymphoid tissues and immune cells (Weigent & Blalock, 1987; McCarthy & Dale, 1988; Benschop et al., 1996; Sternberg, 2006). It is therefore plausible that participating in activities that are associated with a combination of stressors (e.g. sustained military training and routine operations, travelling across time zones for sports competitions, ultra-endurance events, adventure and exploration activities) may amplify or accumulate immune modulating neuroendocrine responses and induce greater perturbations to host defences.

Indeed, military-based field studies have reported depressed cellular and humoral mediated adaptive immunity, depressed oral-respiratory mucosal immunity, increases in pro-inflammatory cytokines and increases in infection rates leading to course failure whilst adhering to multi-stressor combat courses (Martinez-Lopez et al., 1993; Boyum et al., 1996; Gomez-Merino et al., 2003; Gomez-Merino et al., 2005; Tiollier et al.,

2005; Gundersen et al., 2006). It is however difficult to determine which individual or combination of stressors is responsible for these immune perturbations, primarily due to limited research control. Additionally, carbohydrate (CHO) intake during stress stimuli (e.g. exercise) has been reported to attenuate the stress induced immune perturbation (Gleeson, 2006), whilst protein (PRO) may also provide a favourable effect, but this remains to de determined (Walsh et al., 1998). It is additionally plausible that carbohydrate and protein consumed during the recovery period after physiological stress may aid immune recovery.

Neuroendocrine influence of immune responses.

The effects of physiological stress and subsequent increases in stress hormones have previously been linked with depressions in host defences during the post-stress period (Pedersen et al., 1997; Shephard et al., 1998; Walsh & Whitham, 2006). For example, previous studies have reported that prolonged strenuous exercise (e.g. ≥1 h at ≥70% $\dot{V}$O2max) induces alterations in leukocyte trafficking (lymphocytosis and neutrophilia immediately post-exercise, followed by a lymphopenia and bone marrow originated neutrophilia during recovery), decreases lymphocyte proliferation and phagocytic function, and decreases saliva IgA responses during the recovery period (Pederson et al., 1997; Nieman, 1997; Robson et al., 1999; Gleeson & Pyne, 2000). Researchers indicate that these immune perturbations are likely to be attributed to the increased stress hormone responses observed.

Acute periods of physiological stress are associated with increases in plasma stress hormone release, namely cortisol and catecholamines (Opstad et al., 1980; Opstad & Aakvaag, 1981; Ronsen et al., 2001; Majde & Krueger, 2005; Walsh & Whitham, 2006). Both cortisol and catecholamines (e.g. adrenaline and noradrenaline) have the potential to directly and indirectly (through cytokine interaction) modulate circulatory leukocyte trafficking and functional immune responses (McCarthy & Dale, 1988; Hoffman-Goetz & Pedersen, 1994; Sternberg, 2006). This proposal is emphasized by the presence of glucocorticoid and adrenergic receptors on leukocytes and lymphoid tissues (Cupps & Fauci, 1982; Benschop et al., 1996; Felten et al., 1998; Madden, 2003). In addition, stress hormones have previously been labelled as immunosuppressive, affecting circulatory leukocyte maturation, neutrophil function and oral-respiratory mucosal immunity (Khansari et al., 1990; Pederson et al., 1997; Nieman, 1997; Gleeson & Pyne, 2000).

Glucocorticoid administration in vivo has been reported to suppress maturation, differentiation and proliferation of leukocytes, decrease neutrophil phagocytosis, inhibit

the expression of cell-adhesion molecules involved in leukocyte trafficking (e.g. selectin), and inhibit the production and secretion of chemokines, for example IL-8, a predominant chemokine required for neutrophil recruitment and activation (Forslid & Hed, 1982; Cronstein et al., 1992; Scheinman et al., 1995; Williams et al., 1999; Sacedon et al., 1999; Porreca et al., 1999; Sternberg, 2006).

Similarly, in vivo administration of adrenaline (5 µl·L-1 sodium chloride solution) has been shown to decrease circulating lymphocyte subsets through β2 adrenergic receptor activation on lymphocytes (Schedlowski et al., 1993), possibly inducing lymphocyte redistribution into lymphoid and non-lymphoid tissues (Kruger & Mooren, 2007). On the other hand, increases in noradrenaline after cold exposure, were accompanied by reductions in lymphocyte proliferation response to phytohemagglutinin (PHA; Jurankova et al., 1995). Moreover, administration of a 12 µl·L-1 adrenaline solution and a 120 µl·L-1 noradrenaline solution in vivo led to a 72% decrease in neutrophil phagocytosis (Wenisch et al., 1996b). Whilst, 1 µmol of adrenaline to human neutrophils in vitro resulted in a reduced elastase release from N-formyl-methionyl-leucyl-phenylalanine activated neutrophils (Tintinger et al., 2001). The addition of β adrenergic receptor antagonist propranolol prevented the decrease in elastase release. Investigators concluded that the depressive effects of adrenaline on activated neutrophil elastase release was due to down-regulation of neutrophils by increased levels of cAMP (Tintinger et al., 2001). It therefore appears that stress hormones may play a role in modulating leukocyte trafficking and function.

In regards to oral-respiratory mucosal immunity, salivary glands are innervated by both parasympathetic and sympathetic nerve endings. Thus, alterations in catecholamine status have the potential to alter saliva flow and saliva protein secretions (Chicharro et al., 1998; Bosch et al., 2003; Teeuw et al., 2004). For example, reductions in parasympathetic and subsequent increased sympathetic activation is reported to reduce saliva flow rate (Carpenter et al., 1998; Tenovuo, 1998; Teeuw et al., 2004). Additionally, glucocorticoid (e.g. dexamethasone) administration in vivo has been reported to suppress B-lymphocyte cells maturation, differentiation and proliferation, and inhibit the transepithelial transport of IgA; subsequently reducing saliva IgA concentration in rodents (Wira et al., 1990; Sabbadini & Berczi, 1995). Such findings may possibly account for the suppressed saliva IgA responses observed after stress stimuli (Saxon et al., 1978; Forslid & Hed, 1982; Gleeson & Pyne, 2000). It therefore appears that stress hormones may also play a role in modulating saliva IgA responses.

Multi-stressor field studies

Illness incidences are reportedly one of the primary causes of course failure amongst army ranger cadets during a sixty-two day military training course (Martinez-Lopez et al., 1993). Taking this into account, field studies that have observed the effects of sustained military training courses, which included continuous loaded exercise, intermittent sleep-deprivation (1 to 3 h sleep·day-1) and energy-restriction (>80% restriction of daily energy requirements), have reported immune disturbances featuring altered cellular and humoral mediated adaptive immunity, depressed oral-respiratory mucosal immunity and increases in infection rates (Boyum et al., 1996; Gomez-Merino et al., 2005; Gundersen et al., 2006; Martinez-Lopez et al., 1993; Tiollier et al., 2005).

Five to seven days of continuous military combat course (with and without 3 weeks of physical fitness conditioning prior to the combat course), which included energy-restriction and intermittent sleep-deprivation was associated with a circulating leukocytosis, neutrophilia, and lymphopenia with accompanying reductions in T and B-lymphocyte counts (Boyum et al., 1996; Gomez-Merino et al., 2003; Gomez-Merino et al., 2005; Gundersen et al., 2006). In addition, after the combat course, an enhanced neutrophil chemotaxis, granulocyte-macrophage colony stimulating factor (GM-CSF; after 24 h), and lymphocyte blastogenesis response to lipopolysaccharide (LPS), but not to PHA and concanavalin-A (Con-A) was observed (Boyum et al., 1996; Gundersen et al., 2006). Even though the effects of the aforementioned stress protocols may appear to enhance phagocytic migration, the effects of multi-stressor protocols on specific phagocyte function (e.g. neutrophil degranulation) has not previously been determined.

Moreover, three weeks of physical fitness conditioning (repetitive bouts of exercise on consecutive days) prior to multi-stressor combat courses are reported not to alter circulating leukocyte trafficking or function (Gomez-Merino et al., 2003; Gomez-Merino et al., 2005; Tiollier et al., 2005). However, chronic exercise training prior to a multi-stressor combat course may set the scene for amplified or accumulative immune perturbations when compared with initiating a multi-stressor combat course from rest. For example, even though immune perturbations were evident, Boyum et al. (2006) reported no increased incidence of infection during or up to five weeks following five to seven days multi-stressor military training course initiated from rest. Whereas, increased incidences of upper respiratory illness (URI) were reported during and after three weeks of physical fitness conditioning followed by five days multi-stressor combat course (Gomez-Merino et al., 2005; Tiollier et al., 2005), and a 35% reduced in vitro

lymphocyte response to bacterial challenge has been reported after six weeks of basic cadet training (Lee et al., 1992).

Increased incidences of URI have been reported during sustained military operations in the Canadian Arctic winter (St Rose et al., 1972; Sabiston & Livingstone, 1973). Previous studies have also observed decreases in saliva IgA concentration and increased incidences of URI after three weeks of physical fitness conditioning followed by five days multi-stressor combat course, which also included exposure to differing environmental conditions (4 to 25ºC and 34 to 87% relative humidity; Gomez-Merino et al., 2003; Tiollier et al., 2005). Furthermore, Tioller et al. (2005) reported a substantial increase in saliva cortisol after three weeks of physical fitness conditioning (16 nmol·L-1 pre vs. 31 nmol·L-1 post), which remained elevated for one week after cessation of five days multi-stressor combat course (23 nmol·L-1). Similarly, Gundersen et al. (2006) reported substantial increases in plasma cortisol during a continuous multi-stressor ranger course. Conversely, Gomez-Merino et al. (2003; 2005) reported increased plasma noradrenaline and decreased plasma cortisol following three weeks of physical fitness conditioning followed by five days multi-stressor combat course. The investigators attributed the depressed saliva secretory IgA (S-IgA) concentration to increased sympatheticoadrenergic activation, which possibly down-regulated S-IgA translocation (Woof & Mestecky, 2005).

The discrepancies in immune outcomes observed in multi-stressor field studies are possibly due to different protocols, populations (fitness status) and population numbers used. These factors have the potential to expose participants to differing degrees and durations of individual and combined physiological stress between and within studies. A potential limitation within these studies, is the difficulty in determining which individual or combination of physiological stressors are primarily responsible for the alterations in host defences and incidences of infection observed, whether an amplification or accumulative perturbing process is at play with combining stressors, and/or whether a particular physiological stressor counterbalances another to induce a neutral or immune enhancing outcome.

Controlled laboratory studies

A set on controlled laboratory studies was conduced at Bangor University, School of Sport Health & Exercise Sciences, Extremes Research Group, with the purpose of investigating the effects of: 1. one night of total sleep-deprivation on selected immune (circulating leukocyte trafficking, bacterially-stimulated neutrophil degranulation, saliva

IgA) and stress hormone responses at rest and following prolonged strenuous exercise; 2. two nights of total sleep-deprivation with and without energy-restriction on selected immune and stress hormone responses at rest and after passive cold-exposure; 3. passive cold-exposure inducing modest reductions in whole-body core temperature on selected immune and stress hormone responses; and finally, 4. carbohydrate feeding with and without the addition of protein during recovery from prolonged strenuous exercise on selected immune, stress hormone and insulin responses.

The conclusions drawn from this set of well controlled research designs are as follows:

• A 30 h period of total sleep-deprivation does not alter circulating leukocyte trafficking and plasma cortisol response, or compromise bacterially-stimulated neutrophil degranulation and saliva S-IgA responses at either rest or following submaximal or strenuous exercise (Costa et al., 2008).

• Two nights of total sleep-deprivation with and without a 90% energy-restriction does not alter circulating leukocyte counts and plasma stress hormone responses, or compromise bacterially-stimulated neutrophil degranulation and saliva S-IgA responses at rest (Costa et al., 2010).

• Modest whole-body cooling (rectal temperature (Tre) 35.9℃) decreases circulating lymphocyte counts, bacterially-stimulated neutrophil degranulation, and saliva S-IgA responses, possibly induced by noradrenaline response (Costa et al., 2010).

• A 53 h period of total sleep-deprivation, with and without a 90% energy-restriction, prior to modest whole-body cooling (Tre 35.9℃), do es not further perturbate circulating leukocyte trafficking and plasma stress hormone responses, or further compromise bacterially-stimulated neutrophil degranulation and saliva S-IgA responses to modest whole-body cooling (Costa et al., 2010).

• The ingestion of a carbohydrate and protein solution equal to 1.2 g CHO·kg-1BM and 0.4 g PRO·kg-1BM immediately after, but not 1 h after, prolonged strenuous exercise prevents the usual decrease in bacterially-stimulated neutrophil degranulation. However, does not alter circulating leukocyte trafficking, saliva S-IgA and stress hormone responses (Costa et al., 2009a).

• The ingestion of a carbohydrate bolus alone providing 1.2 g CHO·kg-1BM immediately after prolonged strenuous exercise prevents the decrease in bacterially-stimulated neutrophil degranulation, but does not alter circulating leukocyte trafficking, saliva IgA and plasma cortisol responses (Costa et al., 2009b).

• Increases in plasma insulin, rather than attenuated stress hormone responses, is a likely mechanism by which solutions ingested immediately after prolonged strenuous exercise that contain carbohydrate alone, or with the addition of protein, may maintain bacterially-stimulated neutrophil degranulation during the recovery period (Costa et al., 2009a; Costa et al., 2009b).

Further directions with practical relevance

From a practical viewpoint, disturbed sleep patterns, combined with various degrees of energy-restriction, for more than 48 h is a commonly practiced in military, ultra-endurance, adventure and expedition activities. The effect of considerable energy-restriction (>90%) in conjunction with disturbed sleep pattern (intermittent sleep or partial sleep-deprivation) for a more prolonged time period (e.g. ≥ one week) on immune indices has not been determined and warrants further investigation. Future studies should include, for example, assessment of in vivo responses (e.g. delayed-type hypersensitivity, vaccine challenge), and assess the clinical significance of any reductions of immune function on infection incidence and tissue healing.

Military personnel and individuals partaking in ultra-endurance, adventure and expedition activities in cold ambient environments experience cold exposure to a level that induces considerable reductions in core temperature (Tcore). These individuals are further susceptible to cold induced injuries (e.g. frostbite, non-freezing cold injury and upper respiratory tract lesions), which may have clinical significance if immunocompromised (Shephard et al., 1998). The effects of cold-exposure evoking a substantial reduction in Tcore (≤35.0ºC) on circulating leukocyte trafficking, neutrophil function, saliva IgA responses and other immune indices has not been investigated and warrants further investigation. Future studies should assess the effects of varying cold exposure techniques (e.g. water bath, cold air test, moist skin surface) on immune responses, include in-vivo techniques, in clinically hypothermic individuals (Tcore ≤35.0ºC), and assess the clinical significance of any reductions in immune function on infection incidence and tissue healing from specific cold-induced damage. Additionally, further studies should investigate the separate effects of cold air breathing and cold exposure inducing decreases in Tcore, on saliva IgA responses.

Many endurance athletes do not consume sufficient carbohydrates during exercise to avoid immune perturbations, and this may potentially have a clinical (e.g. illness and infection) and performance (e.g. unsustainable training loads, decrements in performance) significance. There is scope in the literature for future research to investigate the effects of different timings and concentrations of carbohydrate ingestion, with and without the addition of protein, on in vitro and in vivo immune function, and assess the clinical significance of any reductions of immune function on infection incidence. Additionally, the effects that different timing and concentrations of post-exercise carbohydrate provisions and fluid volumes have on other aspects of oral-respiratory mucosal immune responses (e.g. salivary lactoferrin, amylase and lysozyme), reported to be modified by exercise, and may respond differently to nutrient availability in the oral cavity (Ljungberg et al., 1997; Chicharro et al., 1998; Ward et al., 2002; Allgrove et al., 2008), warrants further investigation.

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Immune changes potentially associated with upper respiratory symptons in swimmers during a winter training season

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Introduction

The occurrence of immune changes in high-performance sports athletes with possible consequences for health and performance capacity is a known trend, Until recently few studies had found alterations in NK populations related to chronic exposure to regular training. In true rest situation athletes tend to show normal and similar values to those of sedentary and healthy people (Nieman, DC 2000). Although leukocytes have been shown to decrease after intense long duration exercise, 24 hours after they recover to normal numbers (Shephard, Rhind, & Shek, 1994). However it was suggested that moderate training intensity could increase NK cell count and NKCA in trained individuals (Shepard, et al 1994). Nevertheless, periods of heavy training with transitory impaired performance are associated with some innate and acquired immune depression which normally are reversible by tapering (Gleeson & Bishop, 2005).

There are few studies that address the chronic training effects on immune parameters, however the main ones reported are those that show alterations that occur with T cells, and include lower cells count values and diminished proliferative capacity (Gleeson, 2006). In a longitudinal study over a competitive season with high level Australian swimmers, no alterations were found for B or T cells, but a significant decrease on count and percentages of NK cells were noted (Gleeson et al 1995).

Recently the subsets of NK cells were considered based on their prevalence and function . The majority of NK cells (≈ 90%) are NKCD56dim with high cytotoxic capacity, and NKCD56bright (≈10%) with a higher affinity to IL-2 and a larger capacity to produce cytokines, namely IFN-γ, TNF-β, GM-CSF, IL-10, IL-13 after monokine stimulation (Cooper, 2001).

The striking sensivity of NK cells to exercise stress provides strong support that these cells my be implicated as a potential link between regular physical activity and overall health status (Timmons & Cieslaak, 2008)

Objectives

The aim of this study was to see how training load might influence salivary IgA values, the proportion and phenotypic features of circulating T cells subsets in peripheral blood of high competitive swimmers, and the monitoring of the two NK cells subpopulations, NK CD56bright and NK CD56dim and the possible association with the occurrence of URS episodes during a winter swimming season.

Methods

The sample consisted of a group of swimming athletes of high performance level 13 male (17.2 ± 1.3 years, 174.9 height ± 5.8 and 65.8 ± 6.8 kg weight) and 6 female (15.8 ± 0.8 years, height 163.0 ± 9.4 and 54.6 ± 5.0 weight). Training load was monitored by volume and intensity (Mujika et al 1995).

Blood and saliva samples were taken at rest on 4 characteristic moments of the preparation during a sports season (from September to April): before the start of the season and after a rest period of 5 to 6 weeks (t1) after 7 weeks of a period of gradual increase of training load (t2) after 6 weeks of an intense training cycle (t3) and 48 hours after the main competition (t4). Samples were always taken at the same moment of the day, in sitting position. A time lapse of 36 to 48 hours of rest after the last training session or competition was always respected.

Athletes upper respiratory symptoms (URS) episodes were monitored using daily log books (1 episode= repetition of more than two symptoms on consecutive days). A new episode was considered after an interval of 10 days (minimum) (Bishop, 2006). No differences were observed between genders which allowed putting them together for statistical analysis.

Total leukocyte counts and percentage were measured on an automatic counter (Coulter diff TM Analyser,). B, NK, T, γδ T cells, CD4 and CD8 T cells expressing HLA-DR, CD119 and CD126 T cells were determined by flow cytometry (FACSCalibur; BD, San Jose, C.A., USA).The identification and quantification of the two NK cell subsets was done based on the surface density of CD56 phenotype as follows: CD56dim/CD3-/CD8+/- and CD56bright/CD3-/CD8+/-. The expression of IFN-γ receptor CD119 (clone GIR-208; Pharmingen BD, San Diego, C.A., USA) and Granzyme B production was evaluated within the different NK cell subsets after an electronic gating in a lymphocyte region and an acquisition of 20.000 of total events. Data were analysed using “Infinicyt” software program (Cytognos, Spain).

Results are shown as the percentage of positive cells within each cell subset or/and their mean fluorescence intensity (MFI). Salivary IgA concentration was measured using an inhouse ELISA. Spearman rho for correlation analysis and Friedman tests

with "Dunn's Multiple Comparison Test“ was conduct for statistically analysis ($p<0.05$), preventing from not normal distribution and the low number of the sample subjects

Results and Discussion

Upper Respiratory Symptoms (URS)

The highest number of URS was found between t1 and t2 (53,8%) and also in t3 (43% of the athletes) which was concurrent with a flu peak.These were usually preceded by a lowering of the salivary IgA concentration and secretion rates.

In this study we found a mean of 2,1 ± 1,2 Episodes/athlete. The number of URS fall in line with those previously reported in available literature. The Australian average for general population is 2.5/year (Reid, V. L., Gleeson, M., Williams, N., & Clancy, R. L., 2004) and 3.5/year Episodes for athletes (Cox, A. J., Gleeson, M., Pyne, D. B., Callister, R., Hopkins, W. G., & Fricker, P. A. , 2008) (although there were fewer episodes we must consider that this study lasted only 7 months).

Salivary IgA

An inverse correlation between the mean weekly training volume and the mean salivary IgA concentration and secretation rate ($r=-0.15$, $p=0.001$; $r=-0.183$, $p<0.001$ respectively) was found.

A correlation between the pre-season sIgA concentration and the mean weekly sIgA concentration during the whole training season ($r=0.488$, $p=0.03$) was also found as well as an inverse correlation between de mean sIgA concentration registered during the training season and the number of URTI episodes ($p=-0.501$, $p=0.006$).

Leucocytes

During the season any important variation in WBC on PB was not found, which is in agreement with literature however a slight leukocytosis could be observed from the pre-season to the 2nd moment of the study (t2).

Total Lymphocytes

Although most reports of longitudinal studies in sport do not mention significant alterations in lymphocytes our data shows a trend towards a decrease in this leukocyte population, which is significant at the end of the competitive season after a taper period. Probably the 36 hours of rest after training was not enough to recover the normal values of this lymphocyte population, which seems to agree with Gleeson et al (1995) who found a similar decrease in a longitudinal study with swimmers. However a taper period with reduce training volume and 48 hours rest allowed for a recovery to normal NK values (Gleesson & Bishop, 2005).

T and B Lymphocytes

A reduction in both the percentage and the total number of T (CD3+) and B (CD19+) cells was observed at the end of the training season(t4).

CD4+ and CD8+ T Lymphocytes

Although a small decrease in the total number of CD4+ and CD8+ cells between the basal moment and the 2nd moment of evaluation was seen they were not statistically significant. No changes were observed for the CD4+/CD8+ ratio along the training season.

When looking at the added expression of other markers on the CD4+ T cells we found no changes in the percentage or in the mean Intensity fluorescence (MIF) of CD4+HLA-DR cells during the training season. The percentage of CD4+CD25+ decreased along the training season and showed a MIF peak in the 2nd moment of the study. The percentage of cells expressing the IFN-gR (CD4+CD119+) and the expression per cell of the receptor also decreased along the training season.

The expression of the IL-6R (CD126) at cell level, however was increased during the training season when compared to the basal levels. The percentage of CD4+CD126+ cells was also increased in the 2nd and 3rd moments of evaluation.

When looking at the CD8+ cells we found no changes in the percentage of HLA-DR positive cells during the training season but a peak of MIF was observed in the 3rd moment of the study.

The percentage of cells expressing Granzyme B was increased in the 2nd moment but decreased in the 3rd moment while the expression at the cell level was increase in this last moment of the study. Regarding the expression of the IFN-γR and the IL-6R the CD8+ cells followed the same pattern as the CD4+ cells.

Athletes that suffered more URS episodes during the training season had a lower number of regulatory T cells (CD25+) and a higher number of CD4+ T cells expressing the IFN-gR in the 3rd moment of evaluation that corresponded to an increase in the training load intensity.

γδT cells

A decrease from the 1st to the 2nd moment of the study was found for the total number of γδT cells but their percentage did not change during the training season. A decrease in the expression at the cell level for the IFN-gR was also found along the training season. No changes in the HLA-DR expression were found.

NK cells

Our data showed that the number and percentage of NK cells tended to exhibit a decrease in the total NK population specially on moments of intensified training (M3) $p<0.05$.

Looking at the NK subsets the data show a decrease in the cell numbers of the NK^{dim}, subset during the season, which was significant at t(2) corresponding to the first elevation on training volume. Looking at the percentage we found significantly reduced values more pronounced at t(2) and t(3) ($p<0,001$), that did not recover to the initial values at the end of competitive swimming season.

On the contrary the counts and percentage of the NK^{bright} subset showed an elevation at M2 and M3, which correspond to the heavy training mesocycles, remaining significantly elevated at the end of the season after taper and competition.

The NK population is considered the most responsive of the innate immune system, being cytotoxic and producing cytokines against target cells.

The results show a significant decrease in the percentage and number of NK cells, coinciding with periods of increased training load, never recovering to the initial values observed before the start of the season. In periods of more intense training there is a significant reduction of the $NKCD56^{dim}$ subpopulation which has greater cytotoxic capacity. The other subset, the $NKCD56^{bright}$ (≈10%), has a higher affinity to IL-2 and a large capacity to produce cytokines: IFN-γ, TNF-β, GM-CSF, IL-10, IL-13 after monokine stimulation (Cooper, 2001).

A significant increase in the $NKCD56^{bright}$ / $NKCD56^{dim}$ ratio was also found int(2) and t(3).

The expression of CD119 on subset $NKCD56^{dim}$ was investigated as they have a more cytotoxic role and tend to express the IFN-γ receptor. The % of the expression of CD119 and the expression per cell (MIF) is significantly lower during the training season which could denote a lesser citotoxic activity. The high affinity with IL-2, promotes a large production of INF-γ. When activated $NKCD56^{brigt}$ could be as cytotoxic as the $NKCD56^{dim}$ subset (Timmons & Cieslack, 2008)

Although the expression of Granzyme B at the cell level (MIF) did not exhibit significant alterations during the season, the percentage of cells expressing Granzyme B showed an elevation at the more intense training phases of the season when compared to the pre-season values. The elevated production of Granzyme B surrounding t(2) and t(3) corresponded to a higher incidence of URS around this period.

Conclusions

CD4+, CD8+ and γδ T cells down regulated the IFN-γR expression along the training season and increased the IL-6R expression from the pre-season to the 2nd and 3rd moments of the training season. Since the IFN-γ play an important role in activating the cellular adaptative immune response, decreasing it's production by the T cells may impair the activation of the B cells and the production of immunoglobulins (manly IgG2), possibly leading to an increase in susceptibility to disease.

The total number of NK cells in the peripheral blood (PB) fall in response to the most intense phase of training. We conclude that periods of high training load magnitude have a negative impact on innate immune cytotoxicity. However we were not able to confirm the association between URS occurrence and NK cells behaviour.

At the heavy training phases the $NKCD56^{dim}$ subpopulation showed reduced cell numbers, which could represent an impaired cytotoxic activity with consequences to immunity.

The $NKCD56^{bright}$ subset showed the opposite behaviour increasing at M2. This subpopulation is known to exhibits greater ability to produce regulatory cytokines and chemokines. It is also known that the $NKCD56^{bright}$ subset expresses high levels of adhesion molecules namely the CD62L, which facilitates the traffic of these cells to the lymph nodes and sites of inflammation, which could explain their increased mobilization during heavy training phases. This aspect may be related to redistribution phenomenon (Timmons & Cieslack,2008).

During the season there was a significant increase in the $NKCD56^{bright}$ / $NKCD56^{dim}$ ratio. This ratio had been used as a marker of immune depression, since an elevation of this ratio was in parallel with the reduced citolytic NK activity and associated with the fall in cytotoxicity (Suzui et al 2004).

The NK values tended to gradually recover to pre-season, possibly implying an adaptative mechanism. However there may exist, a compensatory mechanism associated with the fall in total NK and the more citotoxic subset, trough the increase in the percentage of the NK subpopulation that produces more regulatory cytokines.

When we compared athletes that didn't get any URTI, with those that had contracted a high number episodes (>3), we found a significant higher % of NK cells expressing Granzyme B ($p<0.05$) in the last the group.

We can argue that the lower % and MIF expression of CD119 on $NKCD56^{dim}$ throughout the season could represent an impaired immune response.

On the other hand it is possible to view this aspect as an immune adaptation mechanism associated with a down regulation of receptors in the presence of high availability of INF-γ produced by other cells, namely $NKCD56^{brigh}$ stimulated by IL-2 .

The most affected subset was the NKCD56^{brigh}. The real meaning of this behaviour, immune depression or adaptation process is still inconclusive.

However it seems possible that athletes that show a higher NKCD56^{bright} /NKCD56^{dim} ratio are more prone to URS. Our data does confirm that alterations in NK subpopulations may occur during training programs.

The main findings of this study point to an impact on immune function of progressive and light training loads at the initial phase of preparation, after long periods of rest, which should be taken into account by the coaches when adopting recovery strategies aimed at reducing the negative impact of training.

Our results also stress the importance of the use of daily logs or other strategies to monitor how athletes are adapting to workload, which could be useful in detecting early signs of difficulties in this process. For example, preventive measures like nutritional supplementation or training load adjustment could be implemented.

Also important is the medical care support at times of intensified training load, allowing for a rapid diagnosis and treatment of the episodes of illness recorded.

In summary we conclude that athletes subjected to long periods of intense training show alterations both in mucosal and systemic immune parameters.

Parameters related to both innate and acquired immunity show alterations after periods of intensified training volume and /or intensity.

Altered values usually return to basal levels after a tapering period.

It is possible that the sum of all these small alterations may compromise resistance to minor infections, like URS, especially during periods of heavy training. The real meaning of this response behaviour, immune depression or adaptation process, remains inconclusive.

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Nutrition and the immune response to exercise

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There is strong evidence that a bout of heavy exercise and a period of heavy training bring about a short-term decrease in immune function. It remains a matter of contention whether the significant decrease in immune function observed in athletes after heavy exercise translates into an increase in infection (e.g. upper respiratory infection). Indeed, it is worth noting that the number of athletes reporting infection symptoms after a heavy bout of exercise or during heavy training remains quite small.
It may be that the temporary lowering of immune function in athletes after heavy exercise serves a protective role by reducing the short-term inflammatory effects of exercise. Nevertheless, it is important that the athlete avoids a chronic state of immune impairment as can happen in a small number of cases. This presentation will cover the effect of nutrition on the immune response to exercise.

The main take-home messages from this presentation are:

- Provided the athlete consumes a well-balanced diet that meets energy needs the intake of macronutrients and micronutrients will be sufficient to maintain immune health

- Even short durations of energy restriction lasting only 1-2 days decrease immune function (e.g. an athlete making weight for competition)

- Carbohydrate ingestion during exercise or shortly after exercise can attenuate the immune impairment

- A high carbohydrate diet will not only optimise athletic performance but may also decrease the immunosuppressive effect of stress hormones

- Athletes on energy restricted diets should consider a low-dose vitamin supplement

Current evidence supporting the use of immunostimulants to prevent or treat common infections in athletes is limited

The Influence of Aerobic Fitness on Age-related Lymphocyte DNA Damage in Humans: some possible underlying mechanisms

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Aging can be defined as an irreversible, progressive, and time dependent decline in the structure and function of humans and animals, resulting from the interaction of genetic and stochastic factors and that lead to the increased susceptibility to disease and risk of death as the person or animalgrows older (Lenaz, D'Aurelio et al. 2000; Ventura, Genova et al. 2002; Figueiredo, Mota et al. 2008).

The main role of the immune system is to control and eliminate foreign organisms and substances in the host body while at the same time recognizes and therefore spars from destruction the molecules (cells and tissues) from oneself. In most elderly humans, the immune system aging is characterized by an increased frequency and severity of infectious diseases and an increased incidence of cancer, chronic inflammatory disorders and an increased failure to recognize self (hence, autoimmune pathology) (Weinert and Timiras 2003; Gruver, Hudson et al. 2007). This decline in immune system with aging seems to be the consequence of the declines in both the generation of new naı¨ve T and B lymphocytes and the functional competence of memory populations (Gruver, Hudson et al. 2007; Hakim and Gress 2007). The mechanisms that underlie these age-related changes are broad and range from defects in the haematopoietic bone marrow to defects in peripheral lymphocyte migration, maturation and function (Gruver, Hudson et al. 2007). Between these mechanisms, the increased oxidative stress with age may explain, at least in part, the increased accumulation of oxidatively damaged proteins (including membrane protein receptors changes to cytokines), membrane fragility, DNA damage, as well as decrease in cells bioenergetics capacity. In fact, it has been widely described in the literature an age related increase of accumulated products of DNA damage, causing progressive enhanced susceptibility to disease and risk of death. Moreover, the age-related increased concentration of DNA-oxidized bases clearly connects oxidative stress with the aging process and supports the concept of the existence of DNA mutation accumulation throughout life in organs and systems, including the immune system.

Although different origins and mechanisms have been proposed to explain the occurrence of DNA mutations, it is possible that mitochondria play an important role in age-related immunodeficiency. Indeed, age-related oxidative damage to mitochondria results in a progressive reduction in mitochondrial bioenergetic capacity, leading to

cellular energy deficits that compromise overall cellular functionality and to an increase in ROS formation and oxidative stress which damage cell macromolecules such as DNA.

Apart from the age influence itself, it is widely described that chronic exercise reduces oxidative stress and damage, both by decreasing ROS production and increasing antioxidant capacity, and that it improves mitochondria efficiency in several organs and systems. In fact, some studies have already described an opposite effect of exercise on some of the undesirable effects of the aging process on cardiovascular system (Ascensao, Magalhaes et al. 2005), on skeletal muscle (den Hoed, Hesselink et al. 2008; Figueiredo, Ferreira et al. 2008), on the brain (Navarro and Boveris 2007) and, more recently, on lymphocytes (Mota et al. 2010). Indeed, although little is known about the hypothetical influence of chronic physical exercise on the age-related oxidative stress condition and associated mitochondrial functionality in immune cells, our results revealed that lymphocyte DNA damage by both strand breaks and FPG sites is closely associated with age and with an increased state of mitochondria H2O2 production (Mota et al., 2010). An elevated aerobic fitness condition seems to play a key role in attenuating DNA-accumulated damage associated with age. This could contribute to a lower damage in membrane proteins receptors in lymphocyte and probably higher lymphocytes functionality in the more active people of different age. Furthermore, considering the importance of DNA damage in the mutagenic, carcinogenic, and aging processes, the contribution of regular exercise to human health promotion seems to become meaningful. Even so, and considering the reported age-related changes, future studies should rely on the thus far weak link between those changes and lymphocyte functionality.

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Affective State and Resilience to Stressful Situations: Psychosocial and Biological Correlates

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Abstract

Sustained stress, major stressful Life Events, social stress and subjectively perceived daily stress can significantly impact psychological and physical well being, A wide range of short and long-term biological effects including immunological, neuroendocrine and neurotransmitter alterations can result from exposure to stressful situations, which also can contribute to precipitate or exacerbate psychological disturbances.

Evidence has been shown that people with more positive affect overall adapt more readily during times of stress (Zautra et al 2005). Furthermore, the dynamic quality of the relationship between positive and negative affect points to a moderating effect of affective state in psychological and biologic individual stress response.

Correlations of Affective State with psychosocial and immunological markers (Figueiredo-Braga et al 2009), point to a link between positive emotional state and reduced risk of physical illness, less damaging stress responses and a resilience to psychological suffering, stimulating the search for biologic and behaviour mediators (Pressman and Cohen 2005, Steptoe et al 2007).

Introduction

Characteristically, different individuals respond to the same emotionally provocative challenge within an extraordinary varied spectrum.

Individual patterns of emotional reactivity mirrored in central circuitry of emotion, influence peripheral biological indices considered relevant to physical health and illness (reviewed in Davison 2004).

Affective style can be defined as a broad range of processes that, either singly or in combination, modulate an individual's response to emotional challenges, dispositional mood or affect relevant cognitive processes. The maintenance of high levels of positive affect and well-being in the face of significant adversity, and the capacity to regulate negative emotion and to decrease the duration of negative affect, are key components

of a resilient affective style (reviwed in Feder et al 2009). A resilient affective style is associated with the perception of stressful events as less threatening and the promotion of adaptative coping strategies with fast recovery in response to negative events. Positive affective state consequently confers resilience to depression, lead to an increase in overall health and a decrease in mortality rates (Lyubomirsky et al 2005). On the contrary, failure to recover adequately from negative events can be a crucial ingredient of vulnerability to mood disorders and physical illness, in relation with sustained elevations in multiple systems activated in response to life changing events (Watson 2005, Pressman and Cohen 2005).

Positive and Negative Affect can be largely independent and Positive Affect may predict healthier physiological processes above and beyond a reduced Negative Affect score (Segerstrom and Sephton 2010).

The association between Positive Affect and psychobiological processes has been established namely through the association with healthier life styles and directly trough the activation of autonomic, neuroendocrine and immune systems (Dockray and Steptoe 2010).

Positive Affect is thought to moderate cardiovascular response to stress (Tugade and Fredrickson 2004), impinge cortisol rhythms and awakening response (Jacobs et al 2007) and has been associated with changes in number of immune cells and with the immune system function (Cohen et al 2006, Marshland et al 2006).

2. **Stress and immunity**

A wide range of short and long-term biological effects including immunological, neuroendocrine and neurotransmitter alterations can result from exposure to stress and stressful events (Anisman et al 2008).

In a sample of 100 women (Figueiredo-Braga et al 2009) psychological assessment included the degree to which the participants appraised their lives as being stressful during the past month (Perceived Stress Scale - PSS), Life Events at long (6 months) and medium-term (1 month) occurrence and impact (Life Experiences Survey - LES), Positive and Negative Affect (PANAS). The participants included 31 depressed psychiatric patients, 25 patients presenting with an autoimmune disease (Systemic Lupus Erythematosus –SLE) as a model for subjects exposed to psychosocial stress in the context of an activated immune system, and 31 age matched controls (Table 1).

In women who were depressed, number of reported Life Events correlated positively with total lymphocyte numbers, CD3, CD4, CD8, CD56. No correlations were detected between Life Events and lymphocyte in physically ill participants (Table 2).

Table 1 – Psychological evaluation

Psychometric measures	SLE patients n=25 1	**Depressed patients** n=31 2	**Control** n=31 3	***p***
HADS D[1]	4.3 (3.4) 1-11	13.1 (3.7) 3-21	2.5 (2.0) 0-6	.000[2] 2>1,3[3]
HADS A	7.7 (4.5) 0-19	14.7 (3.3) 8-20	6.3 (4.0) 0-16	.000 2>1,3
PSS	19.6 (6.7) 4-32	29.4 (4.3) 15-38	14.4 (7.0) 0-31	.000 2>1>3
PANAS P	34.0 (9.1) 16-49	22.0 (6.7) 11-40	36.4 (4.8) 25-47	.000 1,3>2
PANAS N	24.4 (8.7) 14-47	32.8 (4.6) 20-42	19.8 (5.9) 10-31	.000 2>1>3
Life Events 1 month[4]	2.1 (1.3) 1-5	3.1 (2.6) 1-9	3.2 (1.5) 1-5	.464
Life Events 6 months[4]	3.2 (2.4) 1-10	2.6 (2.0) 0-8	2.6 (1.9) 1-9	.546
Life Events 1 month impact[5]	-3.6 (4.8) -15-3	-6.1 (5.8) -19-2	-0.8 (2.8) -4-2	.114
Life Events 6 month impact[5]	-1.3 (6.9) -9-21	-6.2 (5.1) -19-0	-0.5 (4.7) -8-9	.003 2>1,3

[1]Mean (Standard deviation) min-max; [2]Significance level one-way ANOVA; [3]Pos-hoc Analysis using Tukey test, with groups defined as 1-SLE, 2- Depressed, 3-controls; HADS D: Hospital Anxiety and Depression Scale – Depression; HADS A: Hospital Anxiety and Depression Scale – Anxiety; PSS: Perceived Stress Scale; PANAS P: Positive and Negative Affect Schedule – Positive; PANAS N: Positive and Negative Affect Schedule – Negative; [3]nº Life Events occurring last month and last 6 months; [4]Life Events Impact last month and last 6 months;

Table 2 – correlations between Life events and lymphocyte populations

		SLE-nMD patients		**Depressed patients**		**Control**	
		R	*p*	R	*p*	R	*P*
Life Events1 month	Total Lymphocyte	.134	ns	**.505**	**.004**	.198	ns
	CD56	.344	ns	**.410**	**.022**	.047	ns
	CD4	.082	ns	**.410**	**.022**	.144	ns
	CD3	.106	ns	**.471**	**.008**	.150	ns
	CD8	.066	ns	**.369**	**.041**	.038	ns
Life Events 1 month impact	Total Lymphocyte	.185	ns	**-.707**	**.000**	**.668**	**.001**
	CD4	.256	ns	**-.498**	**.004**	.228	ns
	CD3	.118	ns	**-.650**	**.001**	**.379**	**.035**
	CD8	.003	ns	**-.633**	**.000**	.326	ns
	CD19	.238	ns	**-.387**	**.032**	.237	ns

Number of Life Events within previous 6 months correlated negatively with cytokine (IFN-gamma, IL-1beta, IL-2, IL-6, IL-4 and IL-5) levels in depressed patients. Furthermore, a positive association between IFN-gamma, IL-2 and IL-6 levels and the perceived impact of those Life Events was detected uncovering the complex link

existing between immunological markers and life stressors in Depression. Again no correlations were detected in controls or in physically ill subjects.

Curiously, in the present study a positive correlation between cortisol and number and perceived negative impact of life events was found in control subjects. Elevated cortisol levels, regarded as a physiological stress response after severe stressful life events, could mirror adaptative coping strategies in healthy resilient subjects (Cowen 2003, Feder et al 2010). Conversely, in depressed patients, morning cortisol serum level showed to be negatively correlated with Stress, Anxiety and depression severity confirming its relation with depressive disorder.

3. **Positive Affectand immunity**

Positive Affect has been associated with reduced risk of physical illness, contributing to positive well-being and lower mortality (Pressman and Cohen 2005, Steptoe et al 2007). Effects on health measures however showed to be different in men and women and stronger in healthy populations than in those with existing disease (Chida and Steptoe 2008).

In the present study, healthy female control population exhibit significant positive correlation of Positive Affect with Total Lymphocyte, CD3 and CD8 sub populations, pointing to a link between a pleasurable engagement with the environment and immune cell populations.

The influence of Positive Affect on these psychobiological processes was independent from Negative Affect, suggesting that Positive Affect may have characteristic biological correlates.

4. Positive Affect and psychosocial variables

Positive Affect improves well being and promotes a greater capacity to handle stressful situations in diverse situations, since significant life events to medical illness. In our study, Positive Affect was positively correlated with education level in patients with a medical illness – SLE. Higher education was related with lower anxiety, depression and stress symptoms. Reinforcing a positive emotional state and lowering psychological suffering, higher educational attainment could relieve the burden of dealing with a debilitating and unpredictable disease and prevent the occurrence of mood disorders. Furthermore, protective association of positive emotional state with higher cognitive aptitude was not present in healthy or depressed women.

5. **Conclusions**

A relative deficit in positive emotions could increase vulnerability to negative emotions and threat health during stressful times. Thus, the presence of positive emotions may become more critical to preservation of well-being when healthy individuals are submitted to stress. Positive Affect enhances health protective biological responses and shape individual differences in emotional reactivity and regulation. Lymphocyte, cytokine, and hormones may monitor impact of affective state in health, and individual central and peripheral patterns of stress response can be modulated by training and shifted toward a more healthy direction.

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Immune System In Elite Swimmers and Combat Sports Athletes

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Exercise, both high-intensity and prolonged, is a stress to the body that is proportional to the intensity and duration of the exercise, relative to the maximal capacity of the athlete.

Exercise stress leads to a proportional increase in stress hormones, such as cortisol and catecholamines and concomitant changes in several aspects of immunity including neutrophilia; lymphopenia; decreases in granulocyte oxidative burst, NK cell activity, lymphocyte proliferation, and production of cytokines in response to mitogens; and increases in granulocyte and monocyte phagocytosis, and in blood pro and anti-inflammatory cytokines, and nasal and salivary immunoglobulin A (IgA) levels (Bishop, Blannin, Walsh, Robson, & Gleeson, 1999; Mackinnon, 2000; Nieman & Pedersen, 1999). Adrenaline and noradrenaline mediate the acute effects of exercise on lymphocytes, whereas cortisol mediate neutrophyls (Pedersen & Toft, 2000).

Physical exercise is also associated with accelerated reactive oxygen species (ROS) generation (Groussard et al., 2003). As a consequence conditions may be established where ROS production may overwhelm the antioxidant defences and consequently induce damage to macromolecules that may either compromise or stimulate the immune system.

Many aspects of immune function (including cytokine production, mitogen-stimulated lymphocytes proliferation, NK cell cytotoxicity and immunoglobulin secretion) can be depressed temporarily by either a single bout of very severe exercise or a longer period of excessive training (Bishop, Blannin, Walsh, Robson, & Gleeson, 1999; Rowbottom & Green, 2000; Shephard & Shek, 1995; Venkatraman & Pendergast, 2002). Although the disturbance of a single bout of exercise is usually quite transient, it can be sufficient to allow a clinical episode of infection, particularly upper respiratory tract infections (Gleeson, Nieman, & Pedersen, 2004; Shephard & Shek, 1995). However, several papers have described that regular and moderate exercise improves the ability of the immune system to protect the host from infection (Mackinnon, 2000; Peters, 1997). Resting levels of NK cells are enhanced as a result of training (Pedersen & Toft, 2000). Leukocyte number is clinically normal and remains unchanged with training (Mackinnon, 2000).

The response of the immune system to exercise is varied, with different behaviours for each cell type and dependent on the type, intensity and duration of the exercise test and on the training of the subject. Besides these factors, psychological distress and nutritional deficiencies alter immunocompetence and increase the risk of infection. Both heavy exercise and nutrition exert separate influences on immune function. These influences appear to be greater when exercise and poor nutrition act synergistically (Tam, Gomez, Gonzalez-Gross, & Marcos, 2003). Exercise training increases the body requirement for most nutrients. However some athletes adopt an unbalanced dietary regimen predisposing them to immunosuppression (Gleeson & Bishop, 2000). Several elements are known to exert modulatory effects on immune function including zinc, iron, selenium, calcium, copper and magnesium (Beisel, Edelman, Nauss, & Suskind, 1981; Galan, Thibault, Preziosi, & Hercberg, 1992; M.J. Laires & Monteiro, 2001; Mooren, Lechtermann, Fromme, Thorwesten, & Volker, 2001; Shephard & Shek, 1995; Singh, Failla, & Deuster, 1994; Venkatraman & Pendergast, 2002).

Several groups leading investigation in Nutrition and Immunology have shown evidence that magnesium plays a key role in the immune response (Galland, 1988; Perraud, Knowles, & Schmitz, 2004). The reason for assuming an association between magnesium and immune function was based on findings obtained from animal models showing that magnesium deficiency leads to increased inflammation (Libako, Nowacki, Rock, Rayssiguier, & Mazur, 2010; Malpuech-Brugère et al., 1999; Weglicki & Phillips, 1992) with the activation of immune cells, such as monocytes, macrophages and polymorphonuclear neutrophils, that synthesise a variety of biological substances, some of which are powerful inducers of inflammatory events (cytokines, free radicals, eicosanoids) (Rayssiguier, Brussière, Malpuech-Brugère, Rock, & Mazur, 2000; Rayssiguier, Durlach, Gueux, Rock, & Mazur, 1993). Magnesium has also an important role in the inhibition of ROS induced cell injury (Wolf, Trapani, Simonacci, Ferre, & Maier, 2008). Studies conducted in human populations are less extended then those using animal models. Bussiere and co-workers (2002) observed *in vitro* that high magnesium concentrations of the medium decrease human leukocyte activation. Inverse correlations between magnesium intake and C reactive protein plasma levelshave also been observed (Guerrero-Romero & Rodriguez-Moran, 2006; Song, Li, van Dam, Manson, & Hu, 2007).

Swimming is a very popular exercise speciality, frequently for prophylactic purposes. However, due to the exercise environment, performers are often prone to allergies and upper track infections. Competition swimmers are not the exception especially when training seasons are highly demanding. We aimed to evaluate the impact of an acute

maximal exercise and of a 4 month training period of preparation for the swimming national championships on the systemic and mucosal immunity of male and female elite swimmers (Monteiro et al., 2008; Morgado et al., 2008). We observed that leukocytes, lymphocyte subsets and s-IgA rest values were within the reference range. At the beginning of the preparation period, acute exercise induced a significant increase of total lymphocytes, CD3+ (Total T lymphocytes), CD4+ (Th lymphocytes), CD8+ (mainly Tc lymphocytes), CD16+ (natural killer – NK), for the male group but not in the female group. Leukocytosis and an increased number of B cells were also observed after exercise in both groups. The concentrations of serum IgA, salivary IgA and salivary IgA secretory rate were not significant influenced by the acute exercise, in any gender. At rest, during the most intense period of training there was a suppression of NK cells and serum IgA. On the other hand, there was a rise in leukocytes, total lymphocytes and lymphocytes subsets at the end of the season, in the competitive period, after a taper period. These responses were not gender dependent. These findings suggest that a single bout of maximal exercise stimulates systemic cell immunity, and that this response is gender dependent being more pronounced in males then in females. A single bout of maximal exercise did not influence the assessed mucosal immunity. The increment of leukocytes in response to acute exercise has been attributed to the demargination of these cells from the blood vessels wall. This process may result from the increase of blood flow during exercise and consequently enhancement of shear stress; and from the release of catecholamines resulting in their higher concentrations which both accelerate heart rate and reduce the adhesion of leukocytes to the blood vessel. The different magnitude of the response of lymphocytes subpopulations to exercise is in accordance with the described difference in the density of β_2-adrenergic receptors on these cells (Gleeson, 2006). Gender influence on the response of systemic cell immunity to acute exercise may be related to a more relevant increase of catecholamines concentration in males. The chronic effect of intense exercise on leukocytes and lymphocytes rest values is not consensual in the literature and it may be influenced by the sports activity. However these results are consistent with some immune depression at the more intense periods of training which may be reversed after periods of taper, putting to evidence the importance of season preparation by couches.

In combat sports, athletes are divided according to weight. In order to qualify for their respective weight category many athletes undergo impressive weight changes proceeding the competition (Prouteau, Benhamou, & Courteix, 2006). It is also known that sudation and water ingestion reduction are common practices in combat sports to

achieve a target weight. These behaviours are many times associated with severe dehydration (Oppliger, Case, Horswill, Landry, & Shelter, 1996) and electrolyte losses (Jeukendrup & Michael, 2004). Alterations of cellular hydration will influence membrane stretch, membrane bound signalling systems, cytoskeleton, protein phosphorilation, the ionic interior of cell as well as the extent of macromolecular crowding in the cytosol (Haussinger, Lang, & Gerok, 1994) with consequences in the performance of these athletes. Additionally, combat sports frequently involve high impact physical injury. While aiming to study the impact of intense combat sports training (judo, karate and fight) on the immune system of the athletes (Matias, Silva, Monteiro, Raposo, Limão et al., 2008; Matias, Silva, Monteiro, Raposo, Martins et al., 2008) we observed that although there were no significant differences between lymphocytes subpopulations of the athletes and of a sedentary group, there were significantly lower levels of serum haptoglobin and significantly higher levels of serum IgA and salivary TNF-□□as well as significantly higher urinary magnesium in the athletes group. High impact physical injury can lead to intravascular haemolysis and lower haptoglobin values were possibly associated with free haemoglobin capture by haptoglobin and removal of Hp-Hb complex from circulation (Malczewska, Blach, & Stupnicki, 2000). Higher serum IgA and salivary TNF-□□ levels can suggest the existence of chronic low level inflammation (Gleeson, Nieman, & Pedersen, 2004). Athletes' higher urinary magnesium suggests increased magnesium losses in urine as it has been reported in association with prolonged strenuous exercise (Deuster, Dolev, Kyle, Anderson, & Schoomaker, 1987; M. J. Laires & Monteiro, 2007; Meludu et al., 2001).

Prolonged, exhaustive exercise has been shown to be associated with temporary immunosuppression and there is accumulating evidence that this type of exercise may also lead to considerable decreases in some nutrients that may compromise the immune function, especially when accompanied by poor nutrition. Therefore, special efforts should be made to establish the most adequate dose in exercise training and nutrients intake to athletes in order to reach benefits on heath and performance.

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Phenotypic and Functional Characterization of Peripheral Blood Dendritic Cells

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Dendritic cells (DCs) are a minor population of white blood cells that function as professional antigen-presenting cells (APCs) of the immune system highly specialized to capture, process and present antigens to T cells forming a central component of the immune system ideally positioned throughout the entirebody and equipped with a unique capability to transport antigens from the periphery to lymphoid tissues (1-2).

DCs are all bone marrow-derived leukocytes (3). DC progenitors are continuously produced from hematopoetic stem cells andwidely seeded through the blood into nonlymphoid tissues, where they develop to a stage referred to as immature DCs with a high capability for antigen capture and processing, but low T cell stimulatory capability(4-7). In this sense, immature DCs are characterized by a large expression of receptors involved in antigen presentation, such as, MHC class I and class II antigens,(8) Igs Fc receptors,(9) complement receptors (10), c-type lectin receptors (11) like manose receptors,(12-13) langerine (CD207), BDCA-2 (CD303), DEC-205 (CD205) or DC-SIGN (CD209), among others such NOD's receptors family (nucleotide-binding oligomerization domain) and scavenger receptors (CD36, LOX-1 and CD91) (14-16).

DC maturation and migration out of nonlymphoid tissues into the blood or afferent lymph is then promoted by inflammatory mediators (4-7). These migratory cells migrate to secondary lymphoid organs where they home to the T cell areas and become differentiated DCs or mature DCs(17). At this stage, the DCs lost the ability to capture antigen and acquire an increased capacity to stimulate T cells, playing an important role as "sentinels" of the immune system detecting and presenting non-self antigens recognized at peripheral tissues to naive T cells. Thus, it becomes essential for the correct stimulation of T cells that these mature DCs expresses high levels of co-stimulatory molecules like CD40, CD80 and CD86 (18-19).

Moreover, DCs are able to modulate the immune response either by direct contact with other cells or by cytokine secretion. Is this remarkable functional plasticity that determines the outcome of T cell contact, i.e., tolerance or immunity(20-22). In fact, increasing evidence suggests that, depending on their state of maturation and mode of activation, DCs are also essential for the induction and maintenance of peripheral T-

cell tolerance (28-30). Recently, the concept that immature DCs are tolerogenic whereas mature DCs are immunogenic has been challenged by several studies showing that fully mature DCs can induce tolerance and differentiation of regulatory T cells (23-25). In fact, the integration of different signals by the DCs, including antigen dose, cytokine environment at sites of inflammation and encountered pathogen will determine whether DCs will become tolerogenic or immunogenic (23-26).

In human peripheral blood (PB), DCs are recognized on the basis of their high expression of MHC class II antigens in absence of T cells ($TCR^-/CD3^-$), B cells ($CD19^-$), NK cells ($CD56^-$) and monocyte markers ($CD14^-$) (27). Accordingly, it is well established that DCs are not a homogeneous cell population and at least three different DC subsets have been recognized in normal human PB: a $CD11c^+/CD123^{dim}/CD16^-$ myeloid DC subpopulation, a $CD123^{high}/CD33^{-/dim}/CD16^-$ lymphoplasmocytoid subpopulation and a $CD14^{dim}/^-/CD123^{inter}/CD33^{inter}/CD16^+$ monocyte-related subpopulation, previously identified among blood mononuclear cells as a minor subpopulation of monocytes (28-30). Actually, three specific DCs markers can be used to rapidly detect, enumerate, and isolate DCs populations in human PB: BDCA-2, BDCA-4 for $CD11c^-$ $CD123^{bright}$ plasmacytoid DCs and BDCA-3 for $CD11c^+$ $CD123^-$ myeloid DCs. A third population of $CD11c^{bright}$ $CD123^{dim}$ DCs can be identified based on the expression of CD1c (BDC-1) and the lack of B cell lineage antigens (31).

Moreover, human PB DCs as established mediators of immune response to pathogens shape immune response and play a critical role in the understanding pathogenesis of various immune-related diseases(1-2). Therefore, concerning DC potential to either stimulate or inhibit immune responses, they have become an attractive cell type for function as a bio-sensor of the immune system in order to explore insufficient immune responses in infectious diseases and cancer or excessive immune responses in allergy, autoimmunity and transplantation. An increasing number of studies have been made regarding the distribution as well as the phenotypic and functional characteristics of DCs in various immune-related diseases. For example, in HIV-1 infection was demonstrated the existence of significant abnormalities in different subsets of PB DCs, that could help to explain the establishment, dissemination and maintenance of HIV-1 infection, reflecting the occurrence of *in vivo* activation of the immune system.(32) Interestingly, also in system lupus erythematosus, PB DCs exhibit altered activation state and chemokine receptor function. Taken together, these disturbances could lead to an altered distribution of activated DC subsets in peripheral tissues, resulting in immune responses dysregulation and promotion of autoimmunity by decreased induction of self-tolerance (33-34).

Finally, several researches to clarify the relationship between the predisposition to be affected by infections – namely those of the upper respiratory tract (URTIs) – and the participation on specially intensive and voluminous training programs have been focused in the innate immune system. Namely, a recent study from our group, demonstrated a reduced frequency of DCs subsets producing cytokines (IL-1β, IL-6, IL-12, TNF-α and MIP-1β), as well as, of their cytokines amount in response to LPS and IFN-γ stimulation, during a training season.These results support the idea that long term intensive training may affect the function of DCs, reducing their capacity to respond to acute challenges, compromising the response to pathogens.

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Cytokine responses to exercise and gene polymorphisms associated with risk of upper respiratory infections.

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Background: Upper respiratory illness (URI) and the associated symptoms is a significant contributor to reduced performance in high performance athletes, however the aetiology is still uncertain. Post-exercise disturbances in pro- and anti-inflammatory markers differ in illness-prone and healthy athletes and may contribute to the symptoms experienced by some athletes. Single nucleotide changes (polymorphisms) in cytokine genes controlling immune and inflammatory responses and alterations in the associated gene expression may influence the risk for recurrent episodes of URS in athletes.

Aim: The aim of this study was to compare the frequency of cytokine gene polymorphisms between healthy high-performance athletes and athletes prone to frequent URS.

Methods: Cytokine gene polymorphisms were determined in 170 highly-trained athletes participating in studies of immune function associated with recurrent URI. Participants were classified into two groups based on their self-reported number of episodes of URS in the preceding 12 months: Healthy (n=82), 0-2 episodes of URS; Illness-prone (n=88), 3 or more episodes of URS. Polymorphisms in Interleukin(IL)-10 (rs#1800872, 1800896), IL-1 receptor antagonist (rs#419598), IL-6 (rs#1800795), IL-8 (rs#4073), IL-2 (rs#2069762), IL-4 (rs#2243250), Interferon-γ (rs#2430561) were determined using real-time polymerase chain reaction allelic discrimination assays.

Genotypes were classified as high, moderate or low based on established cytokine gene expression. The distribution of genotype frequencies between the two groups of athletes was compared using Pearson's Chi-square (Chi-sq) test. Logistic regression used to model risk for URS as a function of cytokine gene polymorphisms. Odds Ratios (OR) was calculated to predict the risk of frequent URS.

Results: There was a tendency for IL-6 (Chi-sq=5.0, p=0.08) and IL-4 (Chi-sq=4.8, p=0.09) genotype frequencies to differ between groups. The genotype associated with higher IL-6 expression and the genotype associated with lower IL-4 expression appeared more common in the Illness-prone group. The higher IL-6 expression genotype was associated with an increased likelihood of 3 or more URS episodes in a 12 month period (OR: 2.82, 95% CI: 1.01-7.87; p=0.05). The higher IL-2 expression

genotype was associated with a tendency for an increased likelihood of 2 or less URS episodes (OR: 2.64, 95% CI: 0.86-8.09; p=0.09).

Conclusions: The associations of IL-6 high-expression genotype with increased risk of frequent URS and a tendency for IL-2 high expression with reduced risk, supports the possibility that cytokine gene polymorphisms may account in part for individual differences in risk for URS in highly-trained athletes