

## ***Sex differences in immune variables and respiratory infection incidence in an athletic population***

**Michael Gleeson, Nicolette Bishop, Marta Oliveira, Tracey McCauley and Pedro Tauler**

Gleeson, Bishop, McCauley and Oliveira are with the School of Sport, Exercise and Health Sciences, Loughborough University, United Kingdom.

Tauler is with the Department of Fundamental Biology and Health Sciences, University of the Balearic Islands, Palma de Mallorca, Spain

### **ABSTRACT**

*The purpose of this study was to examine sex differences in immune variables and upper respiratory tract infection (URTI) incidence in 18-35 year-old athletes engaged in endurance-based physical activity during the winter months. Eighty physically active individuals (46 males, 34 females) provided resting venous blood samples for determination of differential leukocyte counts, lymphocyte subsets and whole blood culture multi-antigen stimulated cytokine production. Timed collections of unstimulated saliva were also made for determination of saliva flow rate, immunoglobulin A (IgA) concentration and IgA secretion rate. Weekly training and illness logs were kept for the following 4 months. Training loads averaged 10 h/week of moderate-vigorous physical activity and were not different for males and females. Saliva flow rates, IgA concentration and IgA secretion rates were significantly higher in males than females (all  $P < 0.01$ ). Plasma IgA, IgG and IgM concentrations and total blood leukocyte, neutrophil, monocyte and lymphocyte counts were not different between the sexes but males had higher numbers of B cells ( $P < 0.05$ ) and NK cells ( $P < 0.001$ ). The production of interleukins 1 $\beta$ , 2, 4, 6, 8 and 10, interferon- $\gamma$  and tumour necrosis factor- $\alpha$  in response to multi-antigen challenge were not significantly different in males and females (all  $P > 0.05$ ). The average number of weeks with URTI symptoms was  $1.7 \pm 2.1$  (mean  $\pm$  SD) in males and  $2.3 \pm 2.5$  in females ( $P = 0.311$ ). It is concluded that most aspects of immunity are similar in men and women in an athletic population and that the observed differences in a few immune variables are not sufficient to substantially affect URTI incidence. Sex differences in immune function among athletes probably do not need to be considered in future mixed gender studies on exercise, infection and immune function unless the focus is on mucosal immunity or NK cells.*

**Keywords:** exercise training, leukocytes, immunoglobulins, cytokines, illness

---

*Corresponding author:*

Prof Michael Gleeson, School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, Leicestershire LE11 3TU, United Kingdom

Tel. 00 44(0)1509226345, Fax. 00 44(0)1509226301, E-mail: M.Gleeson@lboro.ac.uk

## INTRODUCTION

Resistance to infection is strongly influenced by the effectiveness of the immune system in protecting the host against pathogenic micro-organisms. Within the general healthy human population there is a range of immuno-competency due to genetic differences, age and lifestyle habits. The sex of the individual also affects immune function. In females, oestrogens and progesterone modulate immune function (32) and thus immunity is influenced by the menstrual cycle and pregnancy (21). Consequently, sex-based differences in responses to infection, trauma and sepsis are evident (4). Women are generally more resistant to viral infections and tend to have more autoimmune diseases than men (4). Oestrogens are generally immune enhancing, whereas androgens, including testosterone, exert suppressive effects on both humoral and cellular immune responses. Females have higher levels of plasma immunoglobulin (Ig) than men and exhibit more vigorous responses to exogenous antigens, indicating a higher level of humoral immunity in females than in males (6). In females, there is increased expression of some cytokines in peripheral blood and vaginal fluids during the follicular phase of the menstrual cycle and with use of hormonal contraceptives (7). In the luteal phase of the menstrual cycle, blood leukocyte counts are higher than in the follicular phase (12), mononuclear cell expression of the heterodimeric transcription factor 1 (a key regulator of the innate immune response) is lower (35), and the immune response is shifted towards a T helper (Th) 2-type response (12). The expression of pro-inflammatory and anti-inflammatory genes in response to exercise is also influenced by the menstrual cycle and there are distinct differences in gene expression between women in the luteal phase and men (29). Thus, in the general population, there are differences in some aspects of immune function between men and women that appear to result in women getting fewer viral infections

Prolonged strenuous exercise has been associated with a transient depression of immune function (16, 17) and a heavy schedule of training and competition can lead to immune impairment in athletes. This is associated with an increased susceptibility to infections, particularly upper respiratory tract infections (URTI) (5, 13, 18, 28, 34). However, it is not clear whether any substantial sex differences exist in any aspect of immune function in an athletic population or whether any such differences affect URTI risk.

The aims of the present study were to determine if sex differences exist in resting immune variables including saliva immunoglobulin A (secretory IgA (SIgA)) secretion, plasma immunoglobulin concentrations, numbers of circulating leukocyte and lymphocyte subsets and cytokine production by antigen-stimulated whole blood culture in an athletic population. We also wished to determine if the incidence of URTI was different in male and female athletes during a period of winter training and competition. Our study population was a group of university athletes on a single campus site so that environment and pathogen exposure were likely to be similar for all subjects.

## METHODS

### Subjects

One hundred and eight healthy university students who were engaged in regular sports training (predominantly endurance-based activities such as running, cycling, swimming, triathlon, team games and racquet sports) volunteered to participate in the study. Subjects ranged from recreationally active to Olympic triathletes and their average self-reported training loads ranged averaged 9 h/week. Subjects were required to complete a comprehensive health-screening questionnaire prior to starting the study and had not taken any medication in the 4 weeks prior to the study. All subjects were fully informed about the rationale for the study and of all experimental procedures to be undertaken. Subjects provided written consent to participate in the study, which had earlier received the approval of Loughborough University ethical advisory committee. Subjects were enrolled after having fulfilled all inclusion criteria, and presenting none of the exclusion criteria (determined by both questionnaire and interview).

Subjects could be included if they were currently healthy, had been involved in endurance training for at least 2 years, engaged in at least 3 sessions and at least 3 h of total moderate/high intensity training time per week and were between 18-35 years of age. Subjects representing one or more of the following criteria were excluded from participation: Smoking or use of any medication, abnormal haematology (e.g. erythrocyte or leukocyte counts outside the normal range), suffered from or had a history of cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, haematological or psychiatric illness.

Sample size estimation (14) of 41 subjects per gender group was based on an expected rate of  $2.0 \pm 1.0$  URTI episodes (mean  $\pm$  SD) during the winter months (27), a target difference of 30% in number of episodes (effect size 0.6), statistical power of 80% and a type I error of 5%. We initially recruited 108 volunteers to account for an estimated 25% drop-out rate over the study period. Of these 108 subjects, 50 were female and 58 were male and 80 subjects (34 females, 46 males) completed the study. Their baseline characteristics as shown in Table 1. Self-reported weekly training loads (mean  $\pm$  SD) were similar in males and females ( $9.7 \pm 4.7$  and  $8.7 \pm 3.8$  h/week, respectively,  $P = 0.339$ ). Reasons for dropout were given as foreign travel, injury or persistent illness (preventing subjects from performing training) or due to undisclosed reasons.

### Laboratory visit

The study began in November 2008. Subjects arrived at the laboratory in the morning at 08.30-10:30 following an overnight fast of approximately 12 h. Each subject was asked to empty their bladder before body mass and height were recorded. Information about the study was given to them and they then signed an informed consent form. Subjects then sat quietly for 10 min and completed a health screen questionnaire, training habits questionnaire and inclusion/exclusion criteria questionnaire before providing a saliva sample. With an initial swallow to empty the mouth, unstimulated whole saliva was collected by expectoration into a pre-weighed vial (7 ml-capacity plastic Bijou tubes with screw top) for 2 min

with eyes open, head tilted slightly forward and making minimal orofacial movement. Saliva flow rate (ml/min) was determined by weighing with saliva density assumed to be 1.0 g/ml (9). All saliva samples were stored at  $-20^{\circ}\text{C}$  until analysis. Subsequently, a venous blood sample (11 ml) was obtained by venepuncture from an antecubital vein and blood was collected into two Vacutainer tubes (Becton Dickinson, Oxford, UK) containing either  $\text{K}_3\text{EDTA}$  or heparin. Haematological analysis was immediately carried out on the EDTA sample as detailed below.

### **Questionnaires**

During the 4-month subsequent study period subjects were requested to continue with their normal training programs and they completed a health (URTI symptoms) questionnaire on a weekly basis. Supplements (vitamins and minerals, etc.) were not permitted during this period. Subjects were not required to abstain from medication when they were suffering from illness symptoms but they were required, on a weekly basis, to report any unprescribed medications taken, visits to the doctor and any prescribed medications.

The illness symptoms listed on the questionnaire were: sore throat, catarrh in the throat, runny nose, cough, repetitive sneezing, fever, persistent muscle soreness, joint aches and pains, weakness, headache and loss of sleep. The non-numerical ratings of light, moderate or severe (L, M or S, respectively) of severity of symptoms were scored as 1, 2 or 3, respectively to provide a quantitative means of data analysis (15) and the total symptom score for every subject each week was calculated by multiplying the total number of days each symptom was experienced by the numerical ratings of L, M or S symptoms of 1, 2 or 3, respectively. In any given week a total symptom score  $\geq 12$  was taken to indicate that a URTI was present. This score was chosen as to achieve it a subject would have to record at least 3 moderate symptoms lasting for 2 days or 2 moderate symptoms lasting for at least 3 days in a given week. A single URTI episode was defined as a period during which the weekly total symptom score was  $\geq 12$  and separated by at least one week from another week with a total symptom score  $\geq 12$ . Subjects were also asked to rate the impact of illness symptoms on their ability to train (normal training maintained, training reduced or training discontinued; L, M or S, respectively). Subjects were also asked to fill in a standard short form International Physical Activity Questionnaire (IPAQ; <http://www.ipaq.ki.se/downloads.htm>) at weekly intervals, thus providing quantitative information on training loads in metabolic equivalent (MET)-h/week (11).

### **Blood cell counts**

Blood samples in the  $\text{K}_3\text{EDTA}$  vacutainer (4 ml) were used for haematological analysis (including haemoglobin, haematocrit and total and differential leukocyte counts) using an automated cell-counter ( $\text{A}^{\text{c}}.\text{T}^{\text{TM}}5\text{diff}$  haematology analyser, Beckman Coulter, High Wycombe, UK). The intra-assay coefficient of variation for all measured variables was less than 3.0%.

### **Lymphocyte subsets**

Lymphocyte subsets (CD3, CD4, CD8, CD19, CD56) to enumerate total T cells, T-helper cells, T-cytotoxic cells, B cells and NK cells, respectively were deter-

mined in whole blood samples by three-colour flow cytometry (Becton Dickinson FACS-Calibur) with CellQuest analysis software (Becton Dickinson Biosciences, Oxford, UK) as described previously (25). Forward scatter versus side scatter plots were used to gate on the lymphocyte population by morphology and 10,000 lymphocyte events were acquired per analysis. Estimations of the absolute CD3<sup>+</sup>, CD3+CD4<sup>+</sup>, CD3+CD8<sup>+</sup>, NK cell (CD3-CD56<sup>+</sup>) and B cell (CD3-CD19<sup>+</sup>) numbers were derived from the total lymphocyte count.

### **Monocyte TLR4 expression**

The cell surface expression of toll-like receptor 4 (TLR4) in heparinised whole blood was quantified (geometric mean fluorescence intensity) by flow cytometry as described by Oliveira and Gleeson (31).

### **Antigen-stimulated cytokine production**

Stimulated whole blood culture production of cytokines (IFN- $\gamma$ , tumour necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-2, IL-4, IL-6, IL-8 and IL-10) was determined as follows: 2 ml of heparinised whole blood was added to 2 ml of RPMI medium (Sigma Chemicals, Poole, UK) with added stimulant at a dilution of 1:4000. The stimulant was a commercially available multi-antigen vaccine (Pedi-acel Vaccine, Sanofi Pasteur, UK) containing diphtheria, tetanus, acellular pertussis, poliomyelitis and haemophilus influenzae type b antigens. Whole blood was cultured at 37°C and 5% CO<sub>2</sub> for 24 h. After centrifugation at 1500 g for 10 min at 4°C, supernatants were collected and stored frozen at -80°C prior to analysis of cytokine concentrations using an Evidence Investigator System using the cytokine biochip array EV3513 (Randox, County Antrim, UK). The stimulant dilution of 1:4000 used in this study was based on a previous pilot experiment which established the dose response curve for the measured cytokines over the dilution range of 1:200 – 1:20000. The 1:4000 dilution increased production of all cytokines by at least 4-fold above that of unstimulated whole blood culture, but induced less than 50% of the cytokine production elicited by the highest dose.

### **Plasma immunoglobulins**

The remaining blood in the K<sub>3</sub>EDTA tube was centrifuged at 1500 g for 10 min at 4 °C within 10 min of sampling. The plasma obtained was immediately stored at -80 °C prior to analysis of immunoglobulins A, G and M (immunoturbidometric assay on Pentra 400 autoanalyser, Horiba, France using the manufacturer's calibrators and controls). The intra-assay coefficient of variation for immunoglobulins A, G and M was 3.2%, 1.9% and 2.3%, respectively.

### **Saliva IgA**

Duplicate saliva samples were analysed for SIgA using an ELISA kit (Salimetrics, Philadelphia, USA). The intra-assay coefficient of variation for SIgA was 3.6%. The SIgA secretion rate was calculated by multiplying the SIgA concentration by the saliva flow rate.

### **Statistical Analysis**

Self-reported training load (h/week), average IPAQ scores (MET-h/week), anthropometric and haematological variables were compared between males and

females using unpaired t tests for normally distributed data. The blood leukocyte, neutrophil, monocyte, eosinophil and lymphocyte counts, lymphocyte subset counts, concentrations of secreted cytokines, sIgA concentrations and secretion rates were compared between males and females using unpaired t tests for normally distributed data or nonparametric Mann-Whitney tests for data that were not normally distributed. Statistical significance was accepted at  $P < 0.05$ . Data are expressed as mean  $\pm$  SD.

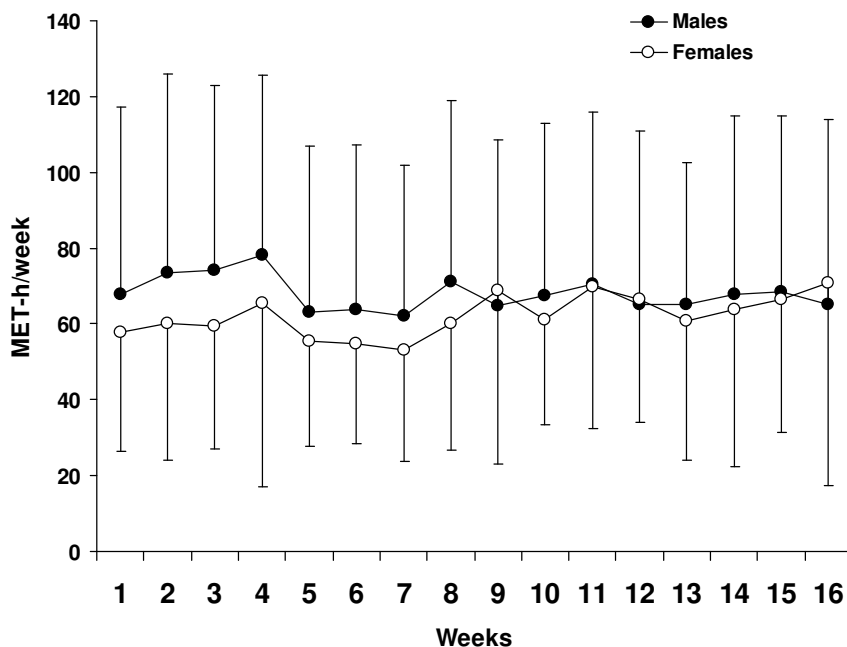
## RESULTS

### Anthropometric and haematological variables

There was no significant difference in age between males and females (Table 1) but males were taller, heavier and had higher BMI than females (all  $P < 0.01$ ). Males had higher RBC count, haematocrit and haemoglobin concentration than females (all  $P < 0.001$ ).

### Training loads

Analysis of the IPAQ questionnaires indicated that the weekly training loads were relatively stable between and within the gender groups over the 4 months of the study (Figure 1) and were equivalent to an average of about 11 h of moderate-vigorous activity per week. The self-reported training loads at the start of the study



**FIGURE 1** – Training loads in MET-h/week over the 4-month study period for men ( $n=46$ ) and women ( $n=34$ ) who completed the study. Data are mean  $\pm$  SD.

and the average IPAQ scores in MET-h/week over the 16 weeks of the study were not significantly different between males and females (Table 1).

**Table 1.** – Anthropometric, training and haematological variables in male and female athletes

	Males (n=46)	Females (n=34)	P
Age (years)	22.9 ± 4.1	22.1 ± 4.0	0.425
Height (cm)	1.81 ± 0.06	1.68 ± 0.06	<0.001
Body mass (kg)	78.0 ± 10.5	62.6 ± 5.8	<0.001
BMI (kg/m <sup>2</sup> )	23.8 ± 2.6	22.3 ± 2.3	0.005
Training load (h/week)	9.7 ± 4.7	8.7 ± 3.8	0.339
IPAQ (MET-h/week)	68.2 ± 39.0	63.4 ± 26.3	0.542
RBC count (x10 <sup>12</sup> /L)	5.01 ± 0.42	4.38 ± 0.38	<0.001
Haematocrit (%)	43.1 ± 2.8	38.7 ± 2.6	<0.001
Haemoglobin (g/L)	146 ± 9	130 ± 10	<0.001

Values are expressed as mean (±SD).

### Plasma immunoglobulins and salivary variables

There were no differences between the sexes for plasma concentrations of IgA, IgG and IgM (Table 2). Saliva flow rates, SIgA concentration and SIgA secretion rates (Table 2) were significantly higher in males than females (all  $P < 0.01$ ). For male and female subjects combined, neither the concentration of SIgA nor its secretion rate were related to the plasma IgA concentration ( $r = -0.122$  and  $r = 0.059$ , respectively; both  $P > 0.05$ ).

**Table 2.** Plasma immunoglobulins and salivary variables in male and female athletes

	Males (n=46)	Females (n=34)	P
Plasma IgA (g/L)	1.52 ± 0.52	1.60 ± 0.50	0.842
Plasma IgG (g/L)	10.16 ± 3.11	10.73 ± 1.71	0.246
Plasma IgM (g/L)	1.40 ± 0.70	1.41 ± 0.70	0.888
Total Ig (g/L)	12.99 ± 3.99	13.74 ± 2.29	0.360
Saliva flow rate (ml/min)	0.50 ± 0.23	0.36 ± 0.20	0.008
SIgA concentration (mg/L)*	180 ± 116	123 ± 53	0.009
SIgA secretion rate (µg/min)*	81.4 ± 55.5	43.8 ± 29.4	<0.001

Values are expressed as mean (±SD). Asterisks indicate data sets that were not normally distributed.

**Blood leukocytes, lymphocyte subsets and monocyte TLR4 expression**

Total blood leukocyte, neutrophil, monocyte and lymphocyte counts were not significantly different (Table 3) but males had higher numbers of B cells ( $P < 0.05$ ) and NK cells ( $P < 0.001$ ) as illustrated in Table 4. Monocyte TLR4 expression tended to be lower in males (geometric mean fluorescence intensity:  $26.1 \pm 13.6$  in females,  $20.5 \pm 12.3$  in males,  $P = 0.062$ ).

**Table 3.** Blood leukocyte counts in male and female athletes

	Males (n=46)	Females (n=34)	P
Leukocyte count ( $\times 10^9/L$ )	$5.66 \pm 1.32$	$5.89 \pm 1.60$	0.477
Neutrophil count ( $\times 10^9/L$ )	$2.71 \pm 1.05$	$3.20 \pm 1.25$	0.062
Monocyte count ( $\times 10^9/L$ )	$0.51 \pm 0.17$	$0.47 \pm 0.14$	0.212
Eosinophil count ( $\times 10^9/L$ )	$0.19 \pm 0.12$	$0.18 \pm 0.13$	0.753
Lymphocyte count ( $\times 10^9/L$ )	$2.17 \pm 0.53$	$1.97 \pm 0.60$	0.127

Values are expressed as mean ( $\pm$ SD).

**Table 4.** Blood lymphocyte subset counts in male and female athletes

	Males (n=46)	Females (n=34)	P
CD3+ cell count ( $\times 10^9/L$ )	$1.28 \pm 0.45$	$1.24 \pm 0.43$	0.718
CD3+CD4+ cell count ( $\times 10^9/L$ )	$0.68 \pm 0.25$	$0.70 \pm 0.23$	0.729
CD3+CD8+ cell count ( $\times 10^9/L$ )	$0.53 \pm 0.28$	$0.48 \pm 0.20$	0.335
CD3-CD19+ cell count ( $\times 10^9/L$ )	$0.23 \pm 0.13$	$0.18 \pm 0.09$	0.048
CD3-CD56+ cell count ( $\times 10^9/L$ )	$0.30 \pm 0.17$	$0.16 \pm 0.07$	<0.001

Values are expressed as mean ( $\pm$ SD).

**Antigen stimulated cytokine production**

The production of interleukins 1 $\beta$ , 2, 4, 6, 8 and 10, IFN- $\gamma$  and TNF- $\alpha$  by multi-antigen stimulated whole blood culture were not significantly different in males and females (Table 5).

**URTI incidence and severity and duration of URTI symptoms**

The average number of weeks with URTI symptoms was  $1.7 \pm 2.1$  in males and  $2.3 \pm 2.5$  in females ( $P = 0.311$ ). For weeks when an URTI episode was present (i.e. total symptom severity score of 12 or more), the mean total symptom severity score was  $22 \pm 7$  and  $22 \pm 11$  in males and females, respectively and the mean duration of symptoms was  $3.6 \pm 1.5$  and  $3.4 \pm 1.5$  days in males and females, respectively.



**Table 5.** Antigen stimulated cytokine production by whole blood culture in male and female athletes.

	Males (n=46)	Females (n=34)	P
<b>IL-1<math>\beta</math> production (pg/ml)*</b>	9.1 $\pm$ 9.9	5.9 $\pm$ 4.8	0.114
<b>IL-2 production (pg/ml)*</b>	140 $\pm$ 227	118 $\pm$ 138	0.996
<b>IL-4 production (pg/ml)*</b>	3.4 $\pm$ 4.1	4.6 $\pm$ 7.6	0.981
<b>IL-6 production (pg/ml)*</b>	167 $\pm$ 133	135 $\pm$ 124	0.375
<b>IL-8 production (pg/ml)*</b>	1178 $\pm$ 738	897 $\pm$ 653	0.133
<b>IL-10 production (pg/ml)*</b>	4.0 $\pm$ 5.3	3.8 $\pm$ 4.6	0.680
<b>IFN-<math>\gamma</math> production (pg/ml)*</b>	31 $\pm$ 59	26 $\pm$ 53	0.431
<b>TNF-<math>\alpha</math> production (pg/ml)*</b>	27 $\pm$ 46	17 $\pm$ 25	0.166

Values are expressed as mean ( $\pm$ SD). Asterisks indicate data sets that were not normally distributed.

## DISCUSSION

The main findings of the present study were that most aspects of immunity are not different between males and female athletes but a few that could potentially influence URTI risk – SIgA concentration and secretion rate, numbers of circulating B cells and NK cells – are lower in women than in men in an athletic population. However, these differences are not sufficient to substantially affect URTI incidence. In contrast monocyte TLR4 expression tended to be higher in females which may compensate for other aspects of their immune function being lower (19). Sex differences in immune function among athletes therefore probably do not need to be considered in future mixed gender studies on exercise, infection and immune function, unless the focus of the study is on mucosal immunity or NK cells.

Low SIgA concentration or secretion rate has been identified as a risk factor for development of URTI in physically active individuals (13, 18, 20, 27). It has been suggested that SIgA levels are a surrogate marker of host protection and the suppression of SIgA after prolonged exercise or heavy training is itself a probable consequence of altered T lymphocyte function (10). Females generally have lower unstimulated saliva flow rates than males (33), whereas SIgA concentration in unstimulated saliva has been reported to be unaffected by sex among relatively large cohorts of healthy young adults (24, 36, 37). A previous small scale study reported lower SIgA concentration and secretion rate in females (n=8) than in males (n=8) among subjects of mixed fitness (3). Two small scale studies on elite swimmers have also reported lower SIgA concentrations in females compared with males (n= 11 females, n = 15 males (18); n = 5 females, n= 7 males (2)); but, to our knowledge, our investigation is the first large scale study to report a sex difference in SIgA secretion in athletes from a range of endurance-based sports. Despite the markedly lower SIgA concentration and secretion rate in females, the

incidence of URTI was not significantly influenced by sex so the clinical significance of the sex difference in SIgA secretion is unclear.

In the general population, women have been reported to have fewer blood monocytes and NK cells, more CD4+ cells and more neutrophils than men (6, 38) and women appear to suffer from fewer viral infections than men (4). Most URTI are of viral origin but in the present study URTI incidence was not significantly different between the sexes. It is possible that the same training load could have a greater depressive effect on humoral immunity (lower SIgA and numbers of circulating B cells) for women than for men (that is not evident in the normal, more sedentary population) but this possibility needs to be resolved by future research. Such an effect may be responsible for the reversal of the usual situation of higher immune function in females into the opposite situation in our athlete cohort. A limitation of the present study is that the phase of the menstrual cycle (when blood and saliva samples were taken) was not determined and we did not establish whether the females were taking oral contraceptives. It is possible that the high training loads of some of the female endurance athletes in our study could have caused them to be amenorrhoeic and one would expect that this would make their immune variables more similar to that of men. This aside, menstrual cycle phase was not found to affect resting SIgA responses in endurance trained female athletes (8).

In healthy normal adults, small differences in single selected markers of immune function may not be clinically important. There are two main reasons for this. Firstly, there is a considerable degree of redundancy in the immune system, such that a small change in the functional capacity of one component of immune function may be compensated for by a change in the functional capacity of another. Secondly, there may be a certain amount of excess capacity in some aspects of immune function, particularly for those functions that are assessed using *in vitro* challenges using a high concentration of stimulant (1). Thus, it cannot be stated with any degree of certainty that small differences in one or more aspects of immune function will influence an individual's susceptibility to infection. Indeed for many aspects of immune function (e.g. blood neutrophil count and oxidative burst activity), it is not even known if the normal variation seen in the healthy adult population is a factor that influences the ability to fight infections (23). More substantial differences in one or more aspects of immune function are probably more likely to affect infection risk although this also depends on the degree of exposure to pathogens and the experience of previous exposure. However, for some immune cell functions a sufficiently large variation or change has been related to altered host defence and susceptibility to disease. For example, some studies indicate that susceptibility to infections and cancer is greater in individuals who possess low NK cell activity compared with individuals with moderate to high NK cell activity (22, 26, 30).

Associations between URTI risk and blood immune parameters have not been extensively examined, though an impaired IFN- $\gamma$  production in unstimulated whole blood culture has been reported in fatigued and illness-prone endurance athletes (10). However, the relevance of this measure of immune function to

infection risk is unclear as cytokine production in the unstimulated state is very low compared with the response to an infectious agent or antigen challenge. Immune functions in females are influenced by endogenous oestrogenic effects (6, 32). In addition, endogenous hormones during the menstrual cycle in female subjects and exogenous hormones in the form of contraceptives or of hormone replacement therapy, affect immune functions such as cytokine production (21), which requires female subjects to be classified as premenopausal (with and without contraceptives) or postmenopausal (with or without hormone replacement therapy). However, Burrows et al. (8) found no differences in SIgA concentration or secretion rate in a group of highly trained female endurance runners over the phases of the menstrual cycle and there was no relationship between SIgA and progesterone concentrations. In the present study the whole blood culture production of measured cytokines in response to multi-antigen challenge was not different in females compared with males. Blood leukocyte, neutrophil, monocyte and lymphocyte counts were also similar in athletic men and women. Circulating numbers of T cells and CD4+ and CD8+ subsets similar as well so it is important to emphasise that most aspects of immunity measured in our study were not different between the sexes. The lower number of circulating B cells and NK cells in females in the present study cannot necessarily be interpreted as meaning lower immune function because it may be that activated cells have moved out of the circulation into the skin, lung, gut, lymph nodes etc. Thus, sex differences in immune function among athletes probably do not need to be considered in future mixed gender studies on exercise, infection and immune function, unless the focus of the study is mucosal immunity or NK cells.

## ACKNOWLEDGEMENTS

This study was sponsored by Yakult Honsha Co., Ltd., Japan and GlaxoSmithKline, UK. Pedro Tauler received a “José Castillejo” grant from the Spanish Ministry of Science and Education.

## REFERENCES

1. Albers R, Antoine JM, Bourdet-Sicard R, Calder PC, Gleeson M, Lesourd B, Samartin S, Sanderson IR, Van Loo J, Vas Dias FW, and Watzl B. Markers to measure immunomodulation in human nutrition intervention studies. *Br J Nutr* 94: 452-481, 2005.
2. Allgrove JE. Factors influencing the mucosal immune response to exercise. PhD thesis, Loughborough University 102-121, 2007.
3. Allgrove JE, Geneen L, Latif S, and Gleeson M. Influence of a fed or fasted state on the s-IgA response to prolonged cycling in active men and women. *Int J Sport Nutr Exerc Metab* 19: 209-221, 2009.

4. Beery TA. Sex differences in infection and sepsis. *Crit Care Nurs Clin North Am* 15: 55-62, 2003.
5. Bishop NC. Exercise and infection risk. In: *Immune function in sport and exercise*, edited by Gleeson M. Edinburgh: Elsevier, 2005, p. 1-14.
6. Bouman A, Schipper M, Heineman MJ, and Faas MM. Gender difference in the non-specific and specific immune response in humans. *Am J Reprod Immunol* 52: 19-26, 2004.
7. Brabin L. Interactions of the female hormone environment, susceptibility to viral infections, and disease progression. *AIDS Patient Care STDS* 16: 211-221, 2002.
8. Burrows M, Bird SR, and Bishop NC. The menstrual cycle and its effect on the immune status of female endurance runners. *J Sports Sci* 20: 339-344, 2002.
9. Chicharro JL, Lucia A, Perez M, Vaquero AF, and Urena R. Saliva composition and exercise. *Sports Med* 26: 17-27, 1998.
10. Clancy RL, Gleeson M, Cox A, Callister R, Dorrington M, D'Este C, Pang G, Pyne D, Fricker P, and Henriksson A. Reversal in fatigued athletes of a defect in interferon gamma secretion after administration of *Lactobacillus acidophilus*. *Br J Sports Med* 40: 351-354, 2006.
11. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, and Oja P. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 35: 1381-1395, 2003.
12. Faas M, Bouman A, Moesa H, Heineman MJ, de Leij L, and Schuiling G. The immune response during the luteal phase of the ovarian cycle: a TH2-type response. *Fertil Steril* 74: 1008-1013, 2000.
13. Fahlman MM, and Engels HJ. Mucosal IgA and URTI in American college football players: A year longitudinal study. *Med Sci Sports Exerc* 37: 374-380, 2005.
14. Faul F, Erdfelder E, Lang A-G, and Buchner A. G\*Power 3: a flexible statistical power analysis program for the social, behavioural and biomedical sciences. *Behav Res Methods* 39: 175-191, 2007.
15. Fricker PA, Pyne DB, Saunders PU, Cox AJ, Gleeson M, and Telford RD. Influence of training loads on patterns of illness in elite distance runners. *Clin J Sport Med* 15: 246-252, 2005.
16. Gleeson M. In: *Immune function in sport and exercise*, edited by Gleeson M. Edinburgh: Elsevier, 2005, p. 67-138.

17. Gleeson M. Exercise and immune function. *J Appl Physiol* 103: 693-699, 2007.
18. Gleeson M, McDonald WA, Pyne DB, Cripps AW, Francis JL, Fricker PA, and Clancy RL. Salivary IgA levels and infection risk in elite swimmers. *Med Sci Sports Exerc* 31: 67-73, 1999.
19. Gleeson M, McFarlin B, and Flynn M. Exercise and Toll-like receptors. *Exerc Immunol Rev* 12: 34-53, 2006.
20. Gleeson M, Pyne DB, Austin JP, Francis JL, Clancy RL, McDonald WA, and Fricker PA. Epstein-Barr virus reactivation and upper respiratory illness in elite swimmers. *Med Sci Sports Exerc* 34: 411-417, 2002.
21. Haus E, and Smolensky MH. Biologic rhythms in the immune system. *Chronobiol Int* 16: 581-622, 1999.
22. Imai K, Matsuyama S, Miyake S, Suga K, and Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet* 356: 1795-1799, 2000.
23. Keil D, Luebke RW, and Pruett SB. Quantifying the relationship between multiple immunological parameters and host resistance: probing the limits of reductionism. *J Immunol* 167: 4543-4552, 2001.
24. Kugler J, Hess M, and Haake D. Secretion of salivary immunoglobulin A in relation to age, saliva flow, mood states, secretion of albumin, cortisol, and catecholamines in saliva. *J Clin Immunol* 12: 45-49, 1992.
25. Lancaster GI, Halson SL, Khan Q, Drysdale P, Wallace F, Jeukendrup AE, Drayson MT, and Gleeson M. Effects of acute exhaustive exercise and chronic exercise training on type 1 and type 2 lymphocytes. *Exerc Immunol Rev* 10: 91-106, 2004.
26. Levy SM, Herberman RB, Lee J, Whiteside T, Beadle M, Heiden L, and Simons A. Persistently low natural killer cell activity, age, and environmental stress as predictors of infectious morbidity. *Nat Immun Cell Growth Regul* 10: 289-307, 1991.
27. Neville V, Gleeson M, and Folland JP. Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. *Med Sci Sports Exerc* 40: 1228-1236, 2008.
28. Nieman DC, Johanssen LM, Lee IW, and Arabatzis K. Infectious episodes in runners before and after the Los Angeles Marathon. *J Sports Med Phys Fitness* 30: 316-328, 1990.
29. Northoff H, Symons S, Zieker D, Schaible EV, Schafer K, Thoma S, Loffler M, Abbasi A, Simon P, Niess AM, and Fehrenbach E. Gender- and menstrual phase dependent regulation of inflammatory gene expression in response to aerobic exercise. *Exerc Immunol Rev* 14: 86-103, 2008.

30. Ogata K, An E, Shioi Y, Nakamura K, Luo S, Yokose N, Minami S, and Dan K. Association between natural killer cell activity and infection in immunologically normal elderly people. *Clin Exp Immunol* 124: 392-397, 2001.
31. Oliveira M, and Gleeson M. The influence of prolonged cycling on monocyte Toll-like receptor 2 and 4 expression in healthy men. *Eur J Appl Physiol* 109: 251-257, 2010.
32. Paavonen T. Hormonal regulation of immune responses. *Ann Med* 26: 255-258, 1994.
33. Percival RS, Challacombe SJ, and Marsh PD. Flow rates of resting whole and stimulated parotid saliva in relation to age and gender. *J Dent Res* 73: 1416-1420, 1994.
34. Peters EM, and Bateman ED. Ultramarathon running and URTI: an epidemiological survey. *S Afr Med J* 64: 582-584, 1983.
35. Schaible E, Boehringer A, Callau D, Niess AM, and Simon P. Exercise and menstrual cycle dependent expression of a truncated alternative splice variant of HIF1 in leukocytes. *Exerc Immunol Rev* 15: 145-156, 2009.
36. Van Anders SM. Chewing gum has large effects on salivary testosterone, estradiol, and secretory immunoglobulin A assays in women and men. *Psychoneuroendocrinol* 35: 305-309, 2010.
37. Van Anders SM. Gonadal steroids and salivary IgA in healthy young women and men. *Am J Human Biol* 22: 348-352, 2010.
38. Willemsen G, Carroll D, Ring C, and Drayson M. Cellular and mucosal immune reactions to mental and cold stress: associations with gender and cardiovascular reactivity. *Psychophysiol* 39: 222-228, 2002.