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Research article

Sex-Based Effects on Immune Changes Induced by a Maximal Incremental Exercise Test in Well-Trained Swimmers

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Abstract

Studies examining the immune response to acute intensive swimming have shown increased leukocytosis and lymphocyte populations. However, studies concerning mucosal immunity and sex differences remain controversial. The objective of the study was to examine sex differences on the immune response to maximal incremental swimming exercise in well trained swimmers. Participants (11 females, controlled for menstrual cycle phase effects; 10 males) performed a maximal incremental 7x200 m front crawl set. Fingertip capillary blood samples were obtained after each 200 m swim for lactate assessment. Venous blood and saliva samples were collected before and 5 minutes after the swimming test to determine total numbers of leukocytes, lymphocytes and subpopulations, and serum and salivary immunoglobulin A (IgA) levels. IgA secretion rate was calculated. Menstrual cycle phase did not influence the immune response to exercise. As for sex differences, exercise induced an increase in leukocytes, total lymphocytes, $CD3^+$, $CD4^+$, $CD8^+$, and $CD16^+/56^+$ in males. In females, only leukocytosis, of a lower magnitude than was observed in males, occurred. $CD19^+$ increased and $CD4^+/CD8^+$ ratio decreased in both groups following exercise whilst IgA, SIgA concentrations, and srIgA did not change. Both males and females finished the incremental exercise very close to the targeted race velocity, attaining peak blood lactate concentrations of 14.6±2.25 and 10.4±1.99 $mmol.L^{-1}$, respectively. The effect of a maximal incremental swimming task on immunity is sex dependent and more noticeable in men. Males, as a consequence of higher levels of immunosurveillance may therefore be at a lower risk of infection than females.

Key words: Swimming, immune system, leukocytes, lymphocytes, IgA.

Introduction

It has been shown that immunocompetence is compromised after intensive exercise, particularly when the latter is accompanied by environmental or competitive stress (Walsh et al., 2011), especially in highly trained athletes (Lopes et al., 2011, Pyne and Gleeson, 1998).

The diversity of exercise protocols, methods of data collection, and the differing levels of conditioning, age, and sex of the study populations in the literature have generated a pool of data that is heterogeneous and hard to analyse.

Some authors reported an elevation in the number of leukocytes (leukocytosis) (McCarthy et al., 1991; McCarthy et al., 1992; Natale et al., 2003; Yamada et al., 2000), a rise in neutrophils (neutrophilia) (Yamada et al., 2000), and elevations in lymphocyte total and subset populations (Natale et al., 2003), both during and immediately after exercise. However, conflicting results e.g. no changes (McKune et al., 2005) or decreases (Pacque et al., 2007) have been reported regarding serum immunoglobulin A concentrations (IgA) after endurance running. As for mucosal immunity, investigations into the salivary IgA concentration (SIgA) and secretion rate (srIgA) responses to acute exercise have also produced divergent findings. As regards SIgA and srIgA levels in males, an increase after high intensity cycling (Blannin et al., 1998, Walsh et al., 2002), no changes after running (Walsh et al., 1999) or after soccer-specific exercises (Sari-Sarraf et al., 2006), and reduced values after endurance running (Pacque et al., 2007) have been reported. In females, neither alterations in SIgA and srIgA after cycling (Fahlman et al., 2001) nor in srIgA after moderate intensity running (Mylona et al., 2002) or after 2 h of rowing (Nehlsen-Cannarella et al., 2000) have been observed. Allgrove et al. (2009) also reported increased SIgA and maintained srIgA levels in a group of active men and women after 2 hours of cycling at 65 % of maximal oxygen uptake. Despite the aforementioned diversity in the acute response of SIgA and srIgA to exercise, it has been found that within 1 h after moderate exercise (Li and Gleeson, 2004) and 3 h following strenuous physical activity (Sari-Sarraf et al., 2006) SIgA values are back to normal.

Sex-based differences in the responses to infection are evident, with women being generally more resistant to viral infections and tending to have more autoimmune diseases than men (Beery, 2003). Few studies, however, have evaluated the influence of sex on the immune response to exercise. Ferrer et al. (2009) reported that both the neutrophil and lymphocyte responses to a swimming session were weaker in females than males. Timmons et al. (2006a) identified greater increases of $CD16^+$ counts in girls than in boys after 60 min of cycling at 70% of maximal oxygen uptake. Timmons et al. (2006b) also observed that exercise-induced increases in lymphocyte and $CD16^+$ counts were greater in older girls versus older boys, but did not differ between young girls and young boys. The same authors also reported greater increases in total leukocyte, lymphocyte, and $CD16^+$ counts in girls than in boys of similar pubertal status. They indicated that

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there is a need to take age, pubertal stage, and sex into account when interpreting the immunological responses to exercise. Other groups have reported that sex has no effect on the response of mucosal immunity (Allgrove et al., 2009; Gleeson et al., 2000; Nieman et al., 2002) to exercise. To our knowledge, no one has addressed the effect of a maximal incremental swimming test on immune system parameters whilst controlling for sex.

Thus, this study aimed to examine sex differences on the response of systemic and mucosal immunity to a maximal incremental swimming set in well-trained national level swimmers.

Methods

Participants

Twenty-one well-trained swimmers (11 females; 10 males) undertaking 13-15 h of pool training and 4 h of dry-land training per week were evaluated at the beginning of a 4-month training cycle. The female participants reported regular menstrual cycles for at least the 4 months prior to participation in this study and were not undergoing oral contraceptive therapy. The testing coincided with the beginning of the follicular phase in four girls and with the luteal phase of the menstrual cycle in seven girls.

Either the study subjects or their parents, as appropriate, were informed about the possible risks of the investigation before they provided their written informed consent to participate. All procedures were approved by the Ethics Committee of the Faculty of Human Kinetics of the University of Lisbon and conducted in accordance with the Declaration of Helsinki for human studies (World Medical Association, 2008).

Maturation

The participants self-assessed their degree of genital organ, breast, and pubic hair development using a questionnaire (Tanner, 1978) accompanied by figures and were then grouped according to pubertal stage.

Body composition measurements

Height (m) and body weight (kg) were assessed (SECA Scale and Stadiometer, Hamburg-Germany), and body mass index (BMI; $kg \cdot m^{-2}$) was calculated. Participants' fat-free mass (FFM) and fat mass (FM) were evaluated using Dual energy X-ray Absorptiometry (DXA) (Hologic Explorer-W, fan-beam densitometer, software QDR for windows version 12.4, Waltham, USA).

Exercise protocol

Athletes were instructed not to consume anything but water after 10 p.m. of the preceding day and to have a minimum of 8 h rest before testing. To standardize pre-exercise food intake and to avoid extending the duration of their fasted state, participants consumed a predefined cereal bar (25 g; 104 kcal) and a glass of water early in the morning of the test. The experimental session took place between 6:30 and 10 a.m. The swimmers performed a standard protocol involving seven 200 m even-paced front crawl swims, graded from easy to maximal intensity, every 5 min, (Pyne et al., 2000). The initial speed was set at 65% of the athlete's best race time for the distance and the target time was decreased by 5 s each time for each subsequent 200 m repetition. The last swim was broken into 4 x 50 m interspersed with 10 s of recovery, to enhance the possibility of the swimmers' achieving maximal intensity. Prior exercise and warm up procedures were standardized for all participants.

Blood and saliva samples

Fingertip capillary blood lactate was determined at the end of each 200 m swim using validated equipment – the Lactate Pro LT-1710 analyser (Arkray KDK).

Venous blood and saliva samples were collected via standard procedures before (pre-exercise) and 5 minutes after (post-exercise) the swimming exercise protocol. Venous blood was collected into tubes containing EDTA and serum separating tubes for plasma and serum separation, respectively.

Whole blood was used for counting of total and subpopulations of lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$, $CD16^+/56^+$ and $CD19^+$) by flow cytometry (FACS Calibur, BD Biosciences) and for haemoglobin concentration and haematocrit quantification (Coulter LH 750, Beckman). Serum was obtained from blood samples by centrifugation and frozen at -80º C for subsequent analysis of IgA by nephelometry (Immage, Beckman Instruments). Post exercise values were corrected for plasma volume variation (Dill and Costill, 1974).

Saliva was collected into pre-weighed salivettes and frozen for later analysis.

SIgA was assessed by enzyme linked immunabsorbent assay (*ELISA)* according to Gomez' procedures (Gomez et al., 1991) and srIgA was calculated (Walsh et al., 2004) in order to account for variation in salivary flow.

Statistical analysis

Data are presented as mean ± standard deviation (mean ± S.D.). ANOVA for repeated measures was used for the assessment of exercise (within participants factor) and sex (between participants factor) effects on immune parameters. The equality of the matrix of variance and sphericity were explored with the Levene *F* test and Mauchly's test, respectively. A paired samples Student's *t* test was used for comparisons between pre and post exercise values in each sex when an interaction between exercise and sex was observed. If variables did not exhibit a normal distribution, pre and post exercise values were compared in each sex using the Wilcoxon signed ranks test (Z). To compare values between menstrual cycle phases, independent samples Student *t*-tests and *Mann-Whitney* tests were used for the variables with and without normal distributions, respectively. Statistical significance was set at $p < 0.05$ in all cases.

Results

The participant's characteristics, including body composition related variables, are presented in Table 1.

7x200 m front crawl swimming test

The last swim of the incremental exercise was performed at a velocity (Vmax) very close to the athlete's target race velocity or personal best race time for the distance, as shown in Table 2.

Table 1. Participants characteristics and body composition. Data are means (±SD).

	Female (n = 11)	Male (n = 10)
Age (years)	16.08 (2.53)	17.41 (1.88)
Height (m)	1.63 (.05)	1.78 (.06)
Weight (kg)	53.4 (5.23)	60.0 (8.80)
BMI (kg·m^{-2})	20.2 (1.13)	21.2 (.93)
FM (%)	23.6 (3.86)	18.5 (6.62)
FFM (kg)	40.4 (2.59)	58.2 (5.56)
Tanner's Stage	n=9 at 2nd state of Adolescence n=2 at Adult state	n=7 at 2nd state of Adolescence n=3 at Adult state

BMI, body mass index; FM, fat mass percentage; FFM, fat free mass; Note: Tanner's Stage: classification of the Maturational State according to Tanner (Tanner, 1978).

Table 2. Evaluated variables in the 7x200m freestyle swimming step test. Data are means (±SD).

	Female (n = 11)	Male (n = 10)
Vmax (m·s^{-1})	1.36 (.07)	1.56 (.11)
% Race Velocity (%)	97.2 (3.45)	98.8 (3.77)
[La] max (mmol·L^{-1})	10.4 (1.99)	14.6 (2.25
Velocity at D-max threshold (m·s^{-1})	1.23 (.05)	1.36 (.07)

Vmax, maximal swimming test speed; % race velocity, relative difference between Vmax and race velocity values in percentage; [La] max, maximal blood lactate concentration.

In some cases Vmax was even higher, which is to be expected since the distance was fractioned and the 10 s interval may constitute an effective recovery time. This fact, along with the maximal blood lactate concentration ([La] max) (Table 2), confirms that the last swim of the incremental exercise was performed at maximal intensity, as required.

Effects of acute exercise on systemic and mucosal immunity

Immune system mean baseline values indicated that the participants were within the Clinical Reference Interval (RI) associated with each variable. However, in the female group mean haematocrit baseline values were below the RI and haemoglobin baseline values were close to the inferior boundary of the RI.

A similar immune response to exercise was observed in the females who were in the follicular phase of their menstrual cycle and those who were in the luteal phase of the menstrual cycle ($p > 0.50$). The aforementioned subsets were therefore assessed as one group (*i.e.* females).

As for sex differences, the acute response to exercise was different between males and females in the case of leukocytes ($F = 20.61$, $p = 0.000$), total lymphocytes ($F = 13.20$, $p = 0.002$), $CD3^+$ ($F = 12.61$, $p = 0.002$), $CD4^+$ ($F = 10.40$, $p = 0.005$), $CD8^+$ ($F = 1.192$, $p = 0.018$) and $CD16^+/56^+$ ($F = 14.02$, $p = 0.002$).

Exercise induced an increase in leukocytes, total lymphocytes, $CD3^+$, $CD4^+$, $CD8^+$, and $CD16^+/56^+$ counts in the male group. In the female group only leukocytosis, of a lower magnitude than was seen in males, was observed with exercise. $CD4^+/CD8^+$ ratio decreased in both groups following exercise (Figure 1).

In the whole group (Figure 1), exercise induced an increase in $CD19^+$ while the concentrations of IgA, SIgA, and srIgA did not change.

Discussion

In the present investigation sex differences in the response of circulating leukocytes, lymphocytes and their subsets to a maximal incremental swimming step test were demonstrated in well-trained swimmers. Exercise induced leukocytosis and lymphocytosis in males but not in females. In female swimmers who were not on oral contraceptives, the response of the systemic and mucosal parameters that were assessed to the swim test was not influenced by menstrual cycle phase.

This finding contrasts with that obtained by Timmons et al. (2005) for 90 min cycling at 65% maximal oxygen uptake i.e. that the lymphocyte response to exercise is greater during the luteal phase than during the follicular phase.

Our salivary IgA results are in accordance with those of other studies in which sex differences were neither identified in the SIgA response to swimming (Gleeson et al., 2000) or endurance running (Nieman et al., 2002), nor in both SIgA and srIgA after 2 hours of cycling exercise at 65 % maximal oxygen uptake (Allgrove et al., 2009).

When the immune response to exercise was considered in males and in females in isolation, various similarities to the results of other investigations, that utilized exercise protocols of equivalent intensity and/or duration, were observed. This was particularly so for males (Natale et al., 2003; Yamada et al., 2000; Zhang et al., 2006). The elevation of total leukocytes, total lymphocytes, and lymphocyte subpopulations as well as the decline in the $CD4^+/CD8^+$ ratio support the results of Kargotich et al. (1997) for elite male swimmers performing an interval training session at 95% of maximal exercise intensity.

As for mucosal IgA, our results are in concordance with other investigations that have demonstrated unchanged SIgA and srIgA values after 5 x 400m front crawl at 85 % of seasonal best time (Dimitriou et al., 2002), or soccer-specific exercises (Sari-Sarraf et al., 2006) in males and after moderate running (Mylona et al., 2002) or a 2 h rowing session (Nehlsen-Cannarella et al., 2000) in females. However, our results for SIgA in both genders contrast with those other investigations that reported a decline after a swimming training session (Gleeson et al., 2000).

The leukocytosis that we observed in the present study may be partly explained by lymphocytosis in the males. However, neutrophilia has been described as one of the most pronounced effects of physical activity on the immune system (Ferrer et al., 2009). Although we did not assess variations in neutrophil count with exercise, the latter cells may have greatly contributed to the increase in leukocyte numbers. An increase in the count of all the lymphocyte subsets that were assessed contributed to the

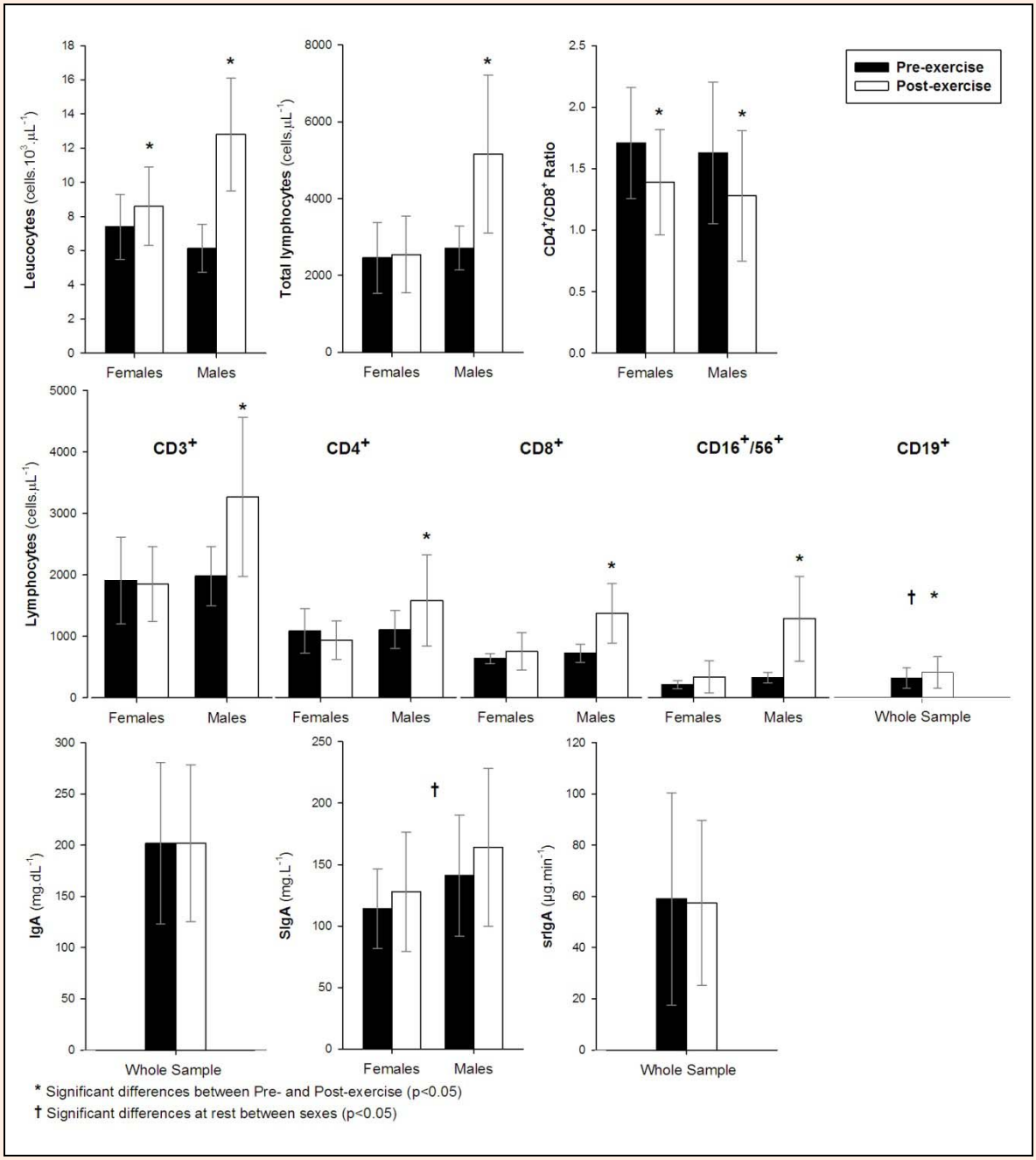

Figure 1. Variation of the number of total leukocyte, total lymphocytes and lymphocyte subsets CD3$^+$ (total T), CD4$^+$ (Th), CD8$^+$ (Tc), CD16$^+$ (NK cells) and CD19$^+$ (B cells) and CD4$^+$/CD8$^+$ Ratio (Th/Tc) in response to acute exercise in female and male national level swimmers.

lymphocytosis that we observed in males. The magnitude of the lymphocyte response to exercise differed between subsets, however, with a higher increase for CD16$^+$ (299 ± 163%), followed by CD8$^+$ (98 ± 84%), CD19$^+$ (44 ± 76%), and finally CD4$^+$ cells (40 ± 44%) being observed. The discrepancy between the relative increases in CD8$^+$ and CD4$^+$ lymphocytes counts, plus the fact that the CD8$^+$ cells count may have been influenced by the NK cells that also express CD8$^+$ (Campbell et al., 2008), is probably one explanation for the observed decrease in the CD4$^+$/CD8$^+$ ratio after swimming. In females the magnitude of B cells increase was not mirrored by lymphocytosis. The decrease of the CD4$^+$/CD8$^+$ ratio may result from the association of both a downward trend of CD4$^+$ (-11 ± 24 %) and an upward trend of CD8$^+$ (40 ± 44 %), despite no alterations having been observed for these subsets of lymphocytes.

Exercise-induced leukocytosis and lymphocytosis has been pointed out to be associated with cell redistribution (Adams et al., 2011) between the circulation and the lungs, the spleen, and muscle. It is thought that the regulation of redistribution of lymphocyte subsets may depend on catecholamine and glucocorticoid concentrations (Gabriel et al., 1992; Shephard, 2003). Our findings are

consistent with those of Nemet et al. (2004) *i.e.* increase (listed in order of magnitude) in NK cells followed by $CD8^+$ and $CD4^+$ upon exercise, possibly related to higher β_2-adrenergic receptor density on the cell surface (Zhang et al. (2006).

Therefore, sex-related differences in the physiological response of some hormones (e.g. catecholamines, cortisol, oestrogen, and testosterone) in association with a differential effect of these hormones and cytokines on lymphocyte subsets can be implicated in the sex-related differences we observed in the response of systemic cell immunity to maximal swimming (Fragala et al., 2011).

The fact that we did not observe a statistically significant difference between pre and post exercise salivary immunoglobulin levels in our study is attributable to the large intra-individual and inter-individual variation in SIgA. This variability has been suggested to be higher in elite swimmers than in sedentary or active individuals (Francis et al., 2005). Acute modifications in SIgA are more likely to be due to alterations in the transport of preformed IgA across the epithelial membrane into the salivary ducts, than to variations in IgA synthesis in the oral mucosa. Autonomic stimulation seems to be responsible for the rise in the delivery of IgA into saliva (Carpenter et al., 2004).

Further investigation involving neutrophil counting and the quantification of the levels of immune system related hormones, particularly catecholamines, cortisol and testosterone, is warranted.

Although it is commonly accepted by the scientific community that exercise elicits lymphocytosis during and immediately after exercise and lymphocytopenia during the recovery from exercise (Walsh et al., 2011), this does not necessarily imply an enhanced immune response during exercise, followed by increased risk of infection or suppressed immunity.

Conclusion

The findings of this study imply that some aspects of the immune response to a maximal swimming exercise are sex dependent. An elevation of leukocytes and lymphocytes subpopulations, more pronounced in males than in females, was confirmed, despite the maintenance of the assessed mucosal immunity. The higher response observed in male swimmers suggests that they may be at a lower risk of infection, due to a higher immunosurveillance.

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All procedures were approved by the Ethics Committee of the Faculty of Human Kinetics of the University of Lisbon and conducted in accordance with the declaration of Helsinki (World Medical Association, 2008). The authors have no conflicts of interest to declare.

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Key points

- Maximal exercise induces an immune response.
- This study investigated the influence of sex over the leukocytes subpopulations and mucosal immune responses to maximal swimming.
- Male swimmers showed a stronger increase of T helper, T cytotoxic and NK lymphocytes than females, suggesting they may be at a lower risk of infection, due to a higher immunosurveillance.
- Mucosal immunity remained unchanged in both sexes.

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