Immune Status and Respiratory Illness for Elite Swimmers During a 12-Week Training Cycle

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The impact of a 12-week training program by elite swimmers on systemic and mucosal immunity was studied prospectively to examine the relationship between changes in immune parameters and the incidence of respiratory illness. Saliva was collected before and after selected training sessions at 2 weekly intervals. There were significant decreases in salivary IgA (p = 0.05) and salivary IgM (p < 0.0001) concentrations after individual training sessions, but no significant changes in salivary IgG or albumin concentrations. Over the 12-week training program there were small but statistically significant increases in pre-exercise concentrations of salivary IgA (p < 0.001), IgM (p = 0.015) and IgG (p = 0.003) and post-exercise salivary IgA (p < 0.001). There were no significant trends over the 12 weeks for any class of serum immunoglobulins but a significant fall in NK-cell numbers (p < 0.001). There were no associations between serum or salivary immunoglobulin levels or NK-cell numbers and upper respiratory tract illness (URTI) during the 12-week program. The data indicated that despite changes in some immune parameters during this final training program prior to competition there were no associations detected with URTI for this cohort of elite swimmers.

Key words: IgA, IgM, IgG, albumin, NK-cells, exercise, infection.

Introduction

Recent reviews of immunity in elite athletes have concluded that there are alterations to various components of the immune system following intensive exercise [2,8,9,18,21,28]. Over the past decade studies have focused on determining the causes of immune suppression in elite athletes and assessing the impact of this phenomenon on the health of athletes undertaking intensive training [2,7 – 9,12,18,20 – 22,28]. The mechanism of the immune suppression, however, remains unclear and reports of any association with infection have been equivocal. The perceptions remain that elite athletes have higher rates of illness during periods of intense training and competitions [12,18,19,22 – 24] and investigations have been directed towards identifying markers for at-risk athletes [10,17,30] in an attempt to reduce the risk of such illnesses. Although several studies have reported increased infection rates associated with exercise-induced mucosal immunosuppression [7,12,20], others have failed to show any association [1,3,4].

This study was designed to investigate the effect of intense exercise on three aspects of the immune system known to influence effective immunity to respiratory illnesses: systemic and mucosal antibodies and cytotoxic NK-cells. In highly trained athletes, across a range of sports, a decrease in salivary IgA concentration following endurance exercise has been a consistent finding [3,4,9 – 14,20,21,30,31]. A study of elite swimmers extended this observation by showing a progressive downward trend in salivary IgA concentration over a seven-month training season [4]. Salivary immunoglobulins, other than IgA, have not been extensively studied, but decreases in salivary IgM concentration have also been reported immediately after intensive exercise [12 – 14]. Taken together these studies indicate that localised defences for the tissues lining the airways of the upper respiratory tract may be more important than changes in systemic antibody production.

While NK-cell numbers and activity increase during exercise, a fall in NK-cell numbers immediately after intensive exercise has been reported [18,21]. A significant fall in NK-cell numbers and percentages has also been observed over a seven-month training season for elite swimmers [4]. It has been suggested that a fall in NK-cells may be associated with an increased risk of viral infection [18] and a possible explanation for the higher URTI rates in elite athletes, however, there is no clear evidence to support this hypothesis.

This prospective study in a cohort of elite swimmers was designed to assess the impact of the final 12-week intensive training program prior to the national championships on measures of systemic (serum immunoglobulins and NK-cells)
and mucosal (salivary immunoglobulins) immunity. The study was designed to clarify issues raised in the previous 7-month study [4] by increasing the frequency of testing from monthly to fortnightly, reducing the length of the study to one cycle of training and eliminating seasonal variations by conducting the study in the summer months. The aim of the study was to reconcile the equivocal findings in the literature and to determine any association between changes in immune parameters during the final intensive training period prior to competition and the incidence of respiratory illness in the athletes.

**Methods**

**Subjects**

The study was conducted with the written informed consent of the Australian Institute of Sport (AIS) swimming team, which consisted of 22 elite swimmers (12 males and 10 females) aged 16–22 years. The athletes were undertaking 10–25 hours of pool training and five hours of dry-land training (resistance, flexibility and circuits) per week in preparation for the Australian Swimming Championships. All procedures were approved by the Ethics Committee of the Australian Institute of Sport.

**Study Design**

The athletes were studied fortnightly, during the final 12-week training cycle in preparation for the Australian Swimming Championships, held in southern hemisphere summer months. Before a scheduled study training session, blood (10 mL) was collected by standard venepuncture technique to enumerate NK-cells and serum immunoglobulins, and saliva (1 mL) was collected for immunoglobulin and albumin quantitation. Saliva (1 mL) was collected again immediately after the training session. Neutrophil oxidative activity was assessed concurrently in these subjects and has been previously reported [25]. All subjects were reviewed by a project team physician at the time of blood sampling for any history of URTI during the study period.

This study was designed to eliminate many of the physiological variables known to cause alterations in salivary immunoglobulin levels [1]. Unstimulated, whole mixed saliva samples were collected at the same time each fortnight, at least one hour post-prandially and a minimum of 18 hours after the previous training session, to reduce the physiological variability of salivary immunoglobulin levels due to diurnal variation, flow rate, dehydration and prior exercise effects. All study sessions were conducted in an indoor heated pool to eliminate any effects of varying temperature (mean air temperature 24 °C, mean water temperature 27 °C).

The phase of training, average distances swum each week and the intensity of training are shown in Fig. 1. The Endurance phase was characterised by high-volume/low-intensity training, the Quality phase by moderate-volume/high-intensity training, the Taper phase by decreasing-volume/high-intensity training, and the Rest phase by low-volume/low-intensity training. The subjective rating of intensity for each training week was determined by the Head Coach and Team Physiologist.

**Serum immunoglobulins**

Serum was prepared from whole blood collected without any anticoagulant. The concentrations of serum IgA, IgG, IgM and albumin were measured by rate nephelometry using a Beckman Array analyser (Beckman, Brea, CA) and Beckman antibodies, controls and calibrators referenced against WHO 67/95. Conversion factor to CRM470 are IgA × 0.99, IgG × 0.96, IgM × 0.95 and albumin × 1.04. The CVs for all proteins were < 3%.

**Salivary immunoglobulins and albumin**

IgA, IgG, IgM and albumin were measured in unstimulated whole mixed saliva by ELISA using commercially prepared IgA-specific antisera (Tago, Burlingame, CA) and IgG, IgM and albumin-specific antisera (Dako immunoglobulins, Glostrup, Denmark). The assays were calibrated with Standard Human Serum referenced against WHO 67/95 (Behringwerke, Marburg, Germany). Conversion factors to CRM470 are IgA × 0.83, IgG × 0.85, IgM × 0.67 and albumin × 1.00. The CVs for all proteins were < 7%.

**NK-cells**

Whole blood (2 mL EDTA) was collected for the full blood count and NK-cell analysis. Total leucocyte count and percentage of lymphocytes were measured on a Coulter JT Haematology Analyser (Coulter Electronics, Heialeah, Florida, USA). Commercial monoclonal antibodies (Coulter) were used to determine numbers and percentages of NK-cells (CD56) by flow cytometry using a Coulter Profile II Flow Cytometer (Coulter Electronics, Heialeah, Florida, USA). The CVs for the NK-cell count were < 10%.
Infections

URTI was defined as symptoms of sore throat, cough, runny nose or fever, and a clinical diagnosis of bacterial or viral throat infections by the team physician. For statistical comparison an athlete’s infection status was classified as either no URTI during the 12-week study period (non-infected) or at least one infection (infected). URTI symptoms lasted from 1 – 3 days. Ten swimmers (45 %) had at least one infection and 12 (55 %) had no infections. Infections were identified in 6/12 (50 %) of male and 4/10 (40 %) of female swimmers over the 12-week period.

Statistical analysis

As the distribution of results were skewed, non-parametric statistics were employed. Pre- and post-exercise concentrations of salivary proteins were compared using the Wilcoxon signed-rank test for paired data on the median subject values. Gender and infection effects were assessed using the Wilcoxon rank-sum test for independent samples on the median subject values. Repeated measures ANOVA was also used on the log transformed salivary protein data to investigate these effects. Changes in salivary proteins and NK cell counts over the study period were assessed using random effects regression models to take into account the multiple observations per subject and appropriately accommodate missing data [27]. A repeated measures analysis of variance [15] was used to test for trends in serum immunoglobulin values over the study period. P-values less than 0.05 were considered significant.

Results

The overall median concentrations for pre- and post-exercise salivary immunoglobulins and albumin are provided in Table 1, and the medians for each week of the study period are presented graphically in Fig. 2. The median NK-cell numbers for each week are provided in Fig 3.

Acute exercise effects

Only salivary immunoglobulins were tested before and after the training sessions. Over the study period the overall median post-exercise salivary IgA and IgM concentrations were significantly lower than the pre-exercise concentrations (Table 1). There were no statistically significant differences between the overall median pre-exercise and post-exercise concentrations.

<table>
<thead>
<tr>
<th>Salivary Protein</th>
<th>Pre-Exercise (n = 95)</th>
<th>Post-Exercise (n = 94)</th>
<th>Session Change (n = 93)</th>
<th>Difference p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (95% CI)</td>
<td>Range</td>
<td>Median (95% CI)</td>
<td>Range</td>
</tr>
<tr>
<td>IgA</td>
<td>55.3 (41.5–71.1)</td>
<td>13.6–177.0</td>
<td>37.1 (29.2–45.9)</td>
<td>8.4–172.0</td>
</tr>
<tr>
<td>IgM</td>
<td>5.7 (5.1–6.6)</td>
<td>1.0–46.2</td>
<td>3.4 (3.0–3.8)</td>
<td>0.4–27.6</td>
</tr>
<tr>
<td>IgG</td>
<td>15.8 (14.7–19.7)</td>
<td>3.2–72.5</td>
<td>13.9 (12.1–17.7)</td>
<td>1.3–56.8</td>
</tr>
<tr>
<td>Albumin</td>
<td>36.0 (32.3–46.0)</td>
<td>7.0–129.0</td>
<td>41.4 (31.5–53.6)</td>
<td>7.8–159.0</td>
</tr>
</tbody>
</table>

Fig. 2 Pre-exercise (○) and post-exercise (△) salivary IgA, IgM, IgG and albumin median concentrations for each week of the 12-week study period.

Table 1 The overall median, 95% confidence interval (CI) and range of concentrations for pre-exercise and post-exercise salivary immunoglobulins (mg/L) and albumin (mg/L). The session change indicates the difference between the pre- and post-exercise salivary concentrations. The significance for the session change differences are indicated (p-value).
of salivary IgG or albumin (Table 1) and no gender or infection status effects for any of the salivary proteins.

Changes over the 12-week training program

Mucosal immunity

There were small but statistically significant increases over the study period (Fig. 2) for pre-exercise salivary IgA (p < 0.001), IgM (p = 0.03) and IgG (p = 0.004) and post-exercise salivary IgA (p < 0.001) and IgG (p = 0.02). The estimated increases per week were 6.7% for both pre- and post-exercise IgA, 3.0% for pre-exercise IgM and 3.5% for both pre- and post-exercise IgG. There were no statistically significant differences according to infection status (Table 2) or gender or changes over the study period for changes from pre-exercise to post-exercise concentration (session change) for salivary immunoglobulins or albumin.

Systemic immunity

There were no significant changes for any of the serum immunoglobulin classes over the 12-week study period and no significant differences in the serum IgA (p = 0.84), IgM (p = 0.24) or IgG (p = 0.73) concentration between infected and non-infected swimmers. The overall median concentration for serum IgA was 1.76 g/L (n = 120; 95% CI = 1.54 – 2.02 g/L; range = 0.49 – 3.0 g/L), serum IgG was 10.40 g/L (n = 120; 95% CI = 9.36 – 11.12 g/L; range = 5.5 – 16.1 g/L) and serum IgM was 1.09 g/L (n = 120; 95% CI = 1.01 – 1.14 g/L; range = 0.51 – 3.2 g/L).

There was a significant decline in NK-cell numbers (p < 0.001) over the 12-week study period (Fig. 3) but no significant differences between infected and non-infected swimmers (Table 2) for NK-cell numbers. The overall median for NK-cell numbers was 0.19 × 10^9 cells/L (n = 104; 95% CI = 0.15 – 0.26; range = 0.05 – 0.45). The overall median percentage of NK-cells was 8.2% of the total leucocyte count (n = 104; 95% CI = 6.7 – 10.4%; range = 2.2 – 15.3%).

Discussion

The results of this study have confirmed previous observations of an acute suppression of salivary IgA and IgM immediately after intensive exercise. Despite this acute post-exercise immunosuppression there were small but statistically significant increases in pre-exercise (resting) levels of all classes of salivary immunoglobulins over the 12-week training program. The intensive exercise of the 12-week training program was also associated with a decline in NK-cell numbers. The study failed to demonstrate any associations between URTI and changes in the immune parameters measured during the 12-week training program.

A significant fall in salivary IgA and IgM concentrations after intensive exercise has been a consistent observation in many studies of athletes [3,4,10 – 14,20,30,31 – 33]. The downward trend in both pre- and post-exercise salivary IgA levels previously reported over a seven-month training season in elite swimmers [4] was not observed in this short 12-week me-

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Table 2  Median, 95% confidence internal (CI) and range of NK-cell counts (× 10^9 cells/L) and pre- and post-exercise concentrations of salivary immunoglobulins (mg/L) and albumin (mg/L) for infected and non-infected swimmers. The significance levels for differences between the infected and non-infected groups are indicated.

<table>
<thead>
<tr>
<th>Immune Parameter</th>
<th>Infected (n = 10)</th>
<th>Non-infected (n = 12)</th>
<th>Difference p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (95% CI)</td>
<td>Range</td>
<td>Median (95% CI)</td>
</tr>
<tr>
<td>NK-Cell Count</td>
<td>0.22 (0.17 – 0.29)</td>
<td>0.02 – 0.73</td>
<td>0.16 (0.11 – 0.22)</td>
</tr>
<tr>
<td>Pre-exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary IgA</td>
<td>71.8 (42.4 – 80.8)</td>
<td>21.6 – 177.0</td>
<td>44.3 (32.1 – 63.3)</td>
</tr>
<tr>
<td>Salivary IgM</td>
<td>6.6 (5.3 – 9.9)</td>
<td>1.0 – 46.2</td>
<td>5.4 (4.8 – 6.0)</td>
</tr>
<tr>
<td>Salivary IgG</td>
<td>17.9 (15.4 – 21.3)</td>
<td>3.5 – 51.6</td>
<td>14.8 (13.0 – 20.1)</td>
</tr>
<tr>
<td>Salivary Albumin</td>
<td>40.2 (32.7 – 53.8)</td>
<td>10.3 – 103.0</td>
<td>33.8 (29.4 – 48.5)</td>
</tr>
<tr>
<td>Post-exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary IgA</td>
<td>43.4 (32.1 – 59.4)</td>
<td>8.4 – 172.0</td>
<td>31.4 (26.7 – 44.6)</td>
</tr>
<tr>
<td>Salivary IgM</td>
<td>3.7 (2.7 – 4.7)</td>
<td>0.4 – 27.6</td>
<td>3.4 (3.1 – 3.8)</td>
</tr>
<tr>
<td>Salivary IgG</td>
<td>13.6 (10.4 – 18.6)</td>
<td>4.5 – 49.2</td>
<td>14.0 (11.7 – 19.5)</td>
</tr>
<tr>
<td>Salivary Albumin</td>
<td>46.4 (34.8 – 74.8)</td>
<td>10.6 – 130.0</td>
<td>38.1 (28.1 – 53.6)</td>
</tr>
</tbody>
</table>
cycle training program. The pre-exercise salivary immunoglobulins all showed small but statistically significant increases over the 12-week program. One of the reasons for this different pattern may have been the selection of the final training cycle prior to a competition. It was noted that the median salivary IgA concentrations at the beginning of the study were at the lower end of the range of concentrations previously observed after months of intense training in a similar cohort of elite swimmers [4,5]. The lower levels in the first two weeks of the study may reflect the accumulative effects of several months of prior intense training. A slight increase in salivary IgA in the taper period prior to competition was also observed in the previous 7-month study [4], suggesting a consistent pattern of recovery of salivary IgA just prior to competition. Alternatively, the short 12-week training period may not have been of sufficient duration to observe the long-term downward trends reported over the 7-month study [4].

Another explanation for the differences may have been the change in coaching regimes for this cohort of elite swimmers to more frequent but less intensive endurance training and shorter training cycles (reduced from 20 to 12 week cycles). Other differences between the two studies of elite swimmers were the seasonal variations at the times of the studies. The former seven-month study [4] was conducted during the autumn-winter months, while the current study was undertaken during summer. These cohorts would have experienced different bacterial/viral challenges with the possibility of different immune responses. The number of infections recorded in the present study was small and not significantly different from a control group monitored over the same period [25].

In addition to our previous 7-month study [4] two other longitudinal studies of salivary immunoglobulin in elite swimmers have been reported. Tharp and Barnes [30] reported a significant decrease in salivary IgA over a three-month training program, while Mackinnon and Hooper [10] reported no significant changes in salivary IgA over a six-month training program. The only other longitudinal study of salivary IgA levels in elite athletes reported a significant increase in pre-training levels over a two-month period in basketball players [29]. A consensus on the longitudinal trends in salivary IgA levels in elite athletes cannot be drawn from the current published studies. Including the current study, differences in the findings of these five longitudinal studies on elite athletes most likely reflect the differences between the sports, the duration, volume and intensity of training regimes, seasonal and other environmental differences and methodological differences.

The acute changes in salivary immunoglobulins and the changes over the final 12-week training cycle showed no correlation with the incidence of URTI in the swimmers during the 12-weeks of the study. The results of the training program undertaken during this study did not result in suppression of salivary IgA levels below which there is a risk of URTI [5]. Although the range of salivary IgA values indicated that some athletes were below the recommended threshold level [5], the median concentrations of both the infected and non-infected groups were both above the recognised risk level.

A decline in NK-cell numbers was observed over the 12-week training program. It is well known that NK-cell numbers and activity increase during exercise and decrease immediately after intensive exercise [9]. NK-cell depletion had been suggested by a previous study to be an effect of long-term intense exercise in elite swimmers [4]. The fortnightly sampling frequency in the current study confirmed that intensive training had an accumulative effect on the decline in NK-cell numbers over time. Although a fall in NK-cell numbers may potentially leave an athlete susceptible to viral infection, there was no association with the URTI reported by the swimmers during this 12-week study.

The neutrophil oxidative activity was also measured during this study [25] and despite suppression during periods of strenuous training there was also no association with URTI in the elite swimmers. The combined results of these studies indicate that despite intensive endurance training inducing suppression of some immune parameters, the risk of infection is low in a well-trained group of elite swimmers. While this study was not designed to assess the “overtrained” athlete the results are consistent with the conclusions of Pyne et al. [24] that only athletes undertaking excessive volumes or intensities of training appear to be at a greater risk of infections. This should give assurance to coaches and athletes that modern coaching regimes may not necessarily be detrimental or associated with a risk of infections due to suppressed immunity.

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