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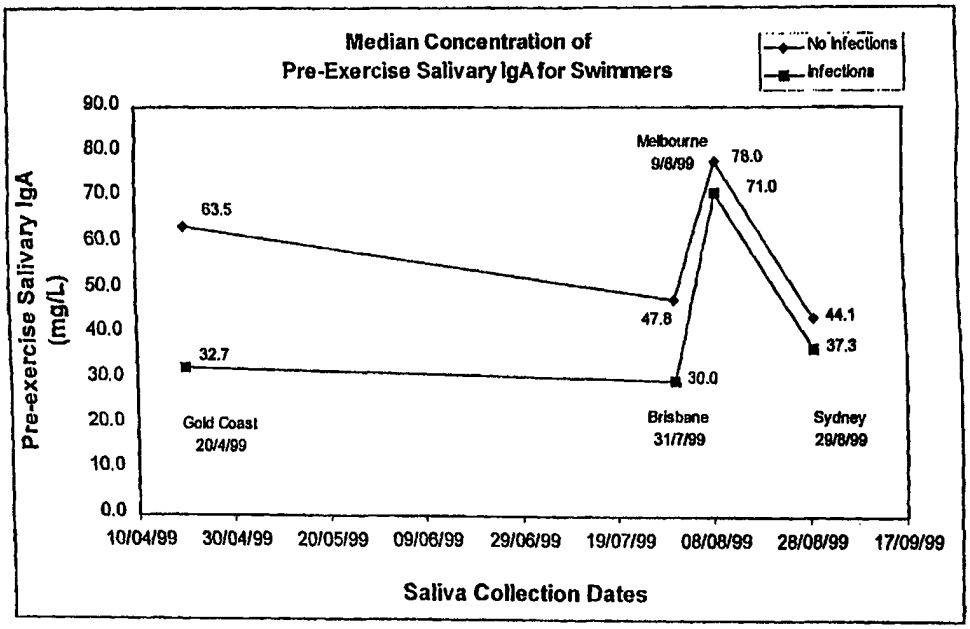


(57) Abstract

The present invention is concerned with methods for determining predisposition to infection in a subject exposed to stressors. In particular the present invention is concerned with methods of assessing the risk of susceptibility to infection in a subject by monitoring levels of IgA and IgA1.

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**TITLE:** PREDISPOSITION TO INFECTION ASSOCIATED WITH INTENSE EXERCISE OR OTHER STRESS

## TECHNICAL FIELD

The present invention is concerned with methods for determining predisposition to infection in a subject exposed to stressors. In particular the present invention is concerned with methods of assessing the risk of susceptibility to infection in a subject by monitoring levels of IgA and IgA1.

## BACKGROUND ART

Secretary IgA (SIgA) is the predominate form of antibody that mediates specific immunological defence at mucosal surfaces (1). Protection is afforded by several recognised mechanisms; interfering with microbial adherence to mucosal surfaces, inhibiting penetration of antigens across the epithelial membrane, complexing with antigens at the basolateral surface of the mucosal epithelium to facilitate elimination by exocytosis into the mucosal lumen, and salvage mechanisms at intracellular and interstitial levels (2). In humans, immunoglobulin A (IgA) occurs as two subclasses that differ in amino acid sequences and glycosylation of the alpha heavy chain (3). IgA1 predominates (approximately 90%) in serum, whereas IgA2 predominates in most mucosal secretions (4). The proportions of the two subclasses vary between mucosal sites due to differences in the distribution of immunoglobulin producing immunocytes (1,5-6). Saliva contains approximately 60% IgA1 in normal adults (4,7-8).

Research into salivary IgA levels in exercising populations has received considerable attention due to reports of a high prevalence of respiratory infections in elite athletes (9-12). The associations between changes in salivary IgA concentrations with exercise are complex and depend on the intensity, duration and periodicity of

training and degree of fitness of the athlete (13-14). Salivary IgA concentrations have been shown to be reduced after intense exercise in the elite athletes training in a variety of endurance sports (14-19). The only report of the influence of exercise on IgA subclasses has been a study of maximal exercise on total IgA and IgA subclasses in human breastmilk (20). In the breastmilk, total IgA and IgA1 but not IgA2 concentrations were decreased after exhaustive exercise, with recovery to baseline levels within 60 minutes (20).

Recently it was reported that low concentrations of IgA in saliva of elite swimmers and a moderately exercising group were associated with an increased risk of respiratory infection (16). Longitudinal studies with elite swimmers have also shown a significant decline in salivary IgA concentrations over a 7-month training season (15-16). However, not all swimmers undertaking the training program were susceptible to infection.

There remains a need for a suitable and timely test to predict susceptibility to infection in subjects exposed to physical or other stress.

It is an object of the present invention to overcome or ameliorate at least some of the disadvantages of the prior art, or to provide a useful alternative.

## SUMMARY OF THE INVENTION

As mentioned previously, IgA includes two subclasses, IgA1 and IgA2. It was unexpectedly found that, of these two subclasses, IgA1 levels in a subject exposed to a stressor are the better indicator of susceptibility to infection. It was also unexpectedly found that the best predictive results using total IgA as an indicator of susceptibility to infection are obtained when IgA is measured in the early training phase. Further, it was

also found that if the decrease in IgA levels which occurs after exposure to a stressor is not recovered rapidly, prolonged reduction in IgA level results. As mentioned above, reduced IgA levels are an indicator of susceptibility to infection. Thus the rate of recovery of IgA levels is also a good indicator of susceptibility to infection.

According to a first aspect, the invention provides a method for assessing potential susceptibility to infection in a subject exposed to a stressor or stressors including:

(a) determination of the subject's immunoglobulin A (IgA) level in a training phase; and

(b) prediction of the subject's susceptibility to infection by comparison of the training phase IgA level with a predetermined threshold value.

According to a second aspect, the invention provides a method for assessing potential susceptibility to infection in a subject exposed to a stressor or stressors including:

(a) determination of the subject's immunoglobulin A1 (IgA1) level; and

(b) prediction of the subject's susceptibility to infection by comparison of the IgA1 level with a predetermined threshold value.

According to a third aspect, the invention provides a method for monitoring a subject's progress following intervention therapy including:

(a) determination of the subject's IgA1 level; and

(b) monitoring the subject's progress by comparison of said IgA1 level with a predetermined threshold value.

According to a fourth aspect, the invention provides a method for assessing the impact of a stressor or stressors on a subject's performance and/or fatigue levels, as defined herein, including:

(a) determination of the subject's salivary IgA1 level; and

(b) assessing the impact of the stressor or stressors on the subject's performance and/or fatigue levels by comparison of said IgA1 level with a predetermined threshold value.

According to a fifth aspect, the invention provides a method for assessing potential susceptibility to respiratory infection in an elite swimmer, including:

(a) determining the swimmer's salivary immunoglobulin A (IgA) level in early training phase; and

(b) predicting the swimmer's potential susceptibility to respiratory infection by comparison of the early training phase IgA level with a predetermined threshold value.

According to a sixth aspect, the invention provides a method for assessing potential susceptibility to respiratory infection in an elite swimmer, including:

(a) determining the swimmer's salivary immunoglobulin A1 (IgA1) level in early training phase; and

(b) predicting the swimmer's potential susceptibility to respiratory infection by comparison of the early training phase IgA1 level with a predetermined threshold value.

According to a seventh aspect, the invention provides a method for assessing a subject's potential susceptibility to infection including:

(a) determining the subject's immunoglobulin A (IgA) level after exposure to a stressor or stressors;

(b) allowing a recovery period as herein defined;

(c) determining the subject's IgA level after the recovery period;

(d) predicting the subject's potential susceptibility to infection by comparison of the IgA level at step (a) above with the IgA level at step (c) above.

According to an eighth aspect, the invention provides a method for assessing a subject's potential susceptibility to infection including:

(a) determining the subject's immunoglobulin A1 (IgA1) level after exposure to a stressor or stressors;

(b) allowing a recovery period as herein defined;

(c) determining the subject's IgA1 level after the recovery period;

(d) predicting the subject's potential susceptibility to infection by comparison of the IgA1 level at step (a) above with the IgA1 level at step (c) above.

Preferably, the immunoglobulin level is determined in early training phase.

Preferably, the infection is a mucosal infection and most preferably, the infection is a respiratory infection.

Preferably, the threshold value is a normal population threshold value. However, in certain instances it may be more appropriate to use an internal personal threshold value.

Preferably, the immunoglobulin is secretory immunoglobulin. Preferably, the secretory immunoglobulin is salivary immunoglobulin. Preferably, the salivary

immunoglobulin is from a sample of whole unstimulated saliva and most preferably the subject is not fasting when the saliva is collected.

Preferably, the subject is an athlete. Preferably, the athlete is an elite swimmer.

Preferably, the stressor is known to influence the efficacy of the immune system.

Preferably, the subject is exposed to a physical and/or psychological stressor. Preferably, the physical and/or psychological stressor is long-term physical training or overtraining.

Preferably, the immunoglobulin level is determined by radial immunodiffusion or ELISA. Preferably, the immunoglobulin level is determined by a rapid near-subject assay. Preferably, the saliva is analysed for the immunoglobulin content *in situ* by contacting an assay device or system with the saliva of a subject. Preferably, the assay is a self-test performed by the subject.

In the context of the present invention, the word "stressor" includes within its meaning but is not limited to physical, physiological, psychological and nutritional stressors which include fatigue.

In the context of the present invention, the word "performance" includes within its meaning performance in relation to other subjects, or performance in relation to personal performance level. Similarly, the term "fatigue" includes within its meaning fatigue in relation to other subjects or fatigue in relation to personal fatigue level.

In the context of the present invention, the word "pre-season" will be understood by those skilled in the art to mean "prior to a return to training".

In the context of the present invention, the words “early training phase” will be understood by those skilled in the art to mean “the first microcycle of training after a rest period”.

In the context of the present invention, the words "late training phase" will be understood by those skilled in the art to mean “the microcycle of training prior to competition”.

While the present invention has been exemplified by examples relating to athletes exposed to a physical stressor, it will be clear to those skilled in the art that similar assessment of susceptibility to infection will apply to other stressful professions and activities eg medical and nursing professions, business and professional travellers, where the stressors may be physical and/or non-physical stressors including physiological and psychological stressors and the like. Further, the present invention will also be applicable to patients exposed to the stress of intervention therapy.

In the context of the present invention, the term "intervention therapy" includes invasive/aggressive medical treatments/procedures including surgical and non-surgical interventions and the like.

In the context of the present invention the term "recovery period" includes a period of rest after exposure to a stressor or stressors. The appropriate length of time for recovery will be determined by the skilled addressee and will vary with the type of stressor and the subject exposed to stress.

Although the present invention has been described predominantly in the context of susceptibility to infection, including reactivation of latent infection, it will be understood

by those skilled in the art that the same principles can be applied to assessing impaired performance and fatigue.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Concentrations (mg/L) of IgA1 (•) and IgA2 (Δ) in saliva samples collected from 25 elite swimmers during early and late phases of a 7-month training season.

Figure 2 Concentrations of IgA1 (mg/L) in the early phase of training for each swimmer and the number of infection episodes recorded during a 7-month training season.

Figure 3 Infection data time points in the Pan Pacs - Salivary IgA/Infection Study

Figure 4 Median concentration of pre-exercise salivary IgA for swimmers

Figure 5 Pre-exercise (o--o) and post exercise (Δ--Δ) salivary immunoglobulin and albumin concentrations (mg/L) for each training session over the 14 day study period.

Figure 6 The percentage recovery of pre-exercise session salivary IgA to the initial level for the day (100%), on days with multiple exercise sessions.

## DESCRIPTION OF THE PREFERRED EMBODIMENT

The concentration of IgA1 in a sample taken from a subject to be tested is determined by radial immunodiffusion (RID) using commercially prepared low-level RID plates and calibrators, ELISA or other technologies applicable to rapid near-subject testing. The present results show that depressed concentrations of IgA1 are particularly associated with a higher risk of infection.

**FIRST STUDY**

EXAMPLE 1

*Subjects and Study Design*

Whole unstimulated saliva was collected from 25 elite swimmers (16 males, 9 females) during the early (April to June) and late (August to October) phases of a 7-month training program (15). The mean time between sample collections for individual athletes was 4 months. The saliva samples were collected prior to the first exercise session on the day of collection and at least 18 hours after the previous exercise session The swimmers aged 16-24 years were undertaking 20-25 hours of pool training and 5 hours of dry-land training per week. Every episode of respiratory infection was recorded during the study and physician-verified (16). The study was conducted with the informed consent of the Australian Institute of Sport (AIS) Swimming Team and had ethics clearance from the Australian Sports Commission.

EXAMPLE 2

*Determination of Total IgA, IgA1 and IgA2*

The concentration of total IgA in whole unstimulated saliva was measured by electroimmuno diffusion (21). The concentrations of IgA1 and IgA2 were determined by radial immunodiffusion (RID) using one batch number of commercially prepared low level RID plates and calibrators. (The Binding Site, Birmingham, UK). The detection limit of the assays was 4.0 mg/L for total IgA, 8.3 mg/L for IgA1 and 7.5 mg/L for IgA2. The between run CVs were 3.5% for total IgA, 4.1% for IgA1 and 3.0% IgA2.

EXAMPLE 3

*Statistical Analysis*

For the purposes of this study the athletes were classified as either having no infections ('healthy') or at least one infection episode during the study period. The Wilcoxon signed rank test for paired data was used to compare ratio values between 'healthy' athletes and athletes who had at least one infection during the study period. Spearman's correlation coefficients were used to determine the association between the number of infections and the concentrations of total IgA, IgA1, IgA2, and the ratio of IgA1:IgA2 in the early and late training phase saliva samples. The Mann-Witney Rank Sum Test was used to compare differences between genders for the percentage of nil-detected values of IgA2. A p-value of $<0.05$ was considered significant.

EXAMPLE 4

*Total IgA, IgA1 and IgA2 Concentrations*

The median concentrations of total IgA, IgA1, IgA2 or the ratio of IgA1:IgA2 were not statistically significantly different between saliva samples collected in the early and late phases of the training program (Table 1). On average IgA1 represented 80% of the total IgA saliva collected in both the early and late phases of the season (Table 1, Figure 1). There were 11 samples with IgA2 concentrations below the detection level of the RID assay; 7 of the early phase samples and 4 of the late phase sample collections. There were no significant differences between gender for the median concentrations of IgA2 or the proportions with nil detected values for IgA2 in either the early or late season samples (Table 2).

**TABLE 1**

The Median (95% Confidence Interval (CI) of the median) and range of salivary IgA concentrations (mg/L) for total IgA, IgA1 and IgA2 in samples collected from elite swimmers in early and late phases of a 7-month training season. The ratio of IgA1 to IgA2 concentrations is presented for all samples with detectable concentrations. The p-value represents the significance of differences between the concentrations and ratios in early and late training phase samples.

| Salivary IgA | Early Training Phase Sample | | | Late Training Phase Sample | | | Significance |
|---|---|---|---|---|---|---|---|
| | n | Median (95% CI) | Range | n | Median (95% CI) | Range | p-value |
| **Total IgA** | 23 | 43.0 (28.5-59.5) | 14-96 | 20 | 45.5 (33.8-55.0) | 25-80 | 0.68 |
| **IgA1** | 23 | 43.0 (31.6-50.7) | 9-102 | 20 | 40.8 (27.5-54.1) | 21-109 | 0.99 |
| **IgA2** | 23 | 8.5 (0.5-14.0) | 0-53 | 20 | 9.3 (5.1-15.0) | 0-47 | 0.64 |
| **IgA1:IgA2** | 16 | 4.0 (2.0-6.0) | 1-15 | 16 | 3.9 (2.9-5.4) | 2-13 | 0.79 |

**TABLE 2**

The number and proportion of male and female swimmers with IgA2 concentrations below the detection limits in saliva samples collected in the early and late phases of a 7-month training season. The p-value represents the significance of differences between genders in each phase of training.

| Training Phase | Male Swimmers | | | Female Swimmers | | | Significance |
|---|---|---|---|---|---|---|---|
| | Total IgA (n) | Low IgA2 (n) | PND* (%) | Total IgA (n) | Low IgA2 (n) | PND* (%) | p-value |
| Early Sample | 14 | 4 | 29 | 9 | 3 | 33 | 0.48 |
| Late Sample | 13 | 3 | 23 | 7 | 1 | 14 | 0.38 |

* Proportion Not Detected

EXAMPLE 5

*Infections*

Seven swimmers (5 males, 2 females) had no episodes of respiratory infection during the 7 month study. The number of infection episodes in the other 18 swimmers ranged from one to seven (Figure 2). The correlations between the number of infections and the concentrations of total IgA, IgA1 and IgA2 (Table 3) showed a statistically significant association between the early season salivary IgA1 concentration and the number of infection episodes in each athlete (Figure 2). The correlation between the early season salivary IgA1 and the number of infections remained significant (p=0.04) even after removing the athlete with the high number of infections (n=7) from the

calculation. There were no statistically significant correlations for other variables (Table 3). Although the ratio of IgA1:IgA2 tended to be lower in swimmers who had no infection episodes during the 7-month training season (Table 4) there were no statistically significant differences in the early or late phase samples collected from athletes who had no infections compared to those who had at least one infection episode.

**TABLE 3**

The Spearman's correlation coefficient (Rho) and significance of the correlations between the number of infections for each swimmer and the concentrations or ratios of total IgA, IgA1 and IgA2 in early and late training phase saliva collections.

| Salivary IgA | Early Training Phase Sample | | Late Training Phase Sample | |
|---|---|---|---|---|
| | Spearman's Rho | p-value | Spearman's Rho | p-value |
| Total IgA | -0.31 | 0.16 | -0.40 | 0.10 |
| IgA1 | -0.51 | 0.01 | -0.08 | 0.76 |
| IgA2 | -0.18 | 0.41 | 0.15 | 0.56 |
| IgA1:IgA2 | 0.20 | 0.46 | -0.01 | 0.98 |

**TABLE 4**

The median and range of ratios of IgA1:IgA2 concentrations in saliva samples collected during early and late phases of training in swimmers with no infections compared with swimmers who had at least one infection during a 7-month training season. The p-value represents the differences between the 'healthy' and infection-prone swimmers.

| Training | No Infection Episodes IgA1:IgA2 Ratio | | | At Least One Infection IgA1:IgA2 Ratio | | | Significance |
|---|---|---|---|---|---|---|---|
| | n | Median | Range | n | Media n | Range | p-value |
| Early Sample | 4 | 2.6 | 2-6 | 12 | 4.1 | 1-15 | 0.25 |
| Late Sample | 3 | 3.9 | 3-4 | 13 | 3.9 | 2-13 | 0.84 |
| Total | 7 | 3.2 | 2-6 | 25 | 4.1 | 1-15 | 0.24 |

The results of this study of elite swimmers indicate that low concentrations of IgA1 below 25 mg/L (Figure 2) in saliva collected early in a 7-month training season were selectively associated with a higher risk of respiratory tract infection episodes during the season. A previous study of respiratory infections in elite swimmers revealed that pre-season concentrations of total IgA below 35 mg/l were associated with high numbers of infection episodes (16). In the current study the saliva samples were

collected early in the training season but not prior to the commencement of the training season.

IgA1 represented approximately 80% of the total IgA concentration in this cohort of elite swimmers. In studies of normal non-exercising adults the proportion of IgA1 in saliva is approximately 60% (4,7-8). IgA1 antibodies are primarily produced in response to protein antigens while IgA2 antibodies are induced by carbohydrates and lipid-containing antigens (1). The fact that IgA1 proteases produced by most mucosal pathogens can degrade IgA1 antibodies, while IgA2 antibodies are protected (1) is of importance when considering resistance to infections at mucosal surfaces. As IgA1 is more susceptible to the proteases produced by mucosal pathogens (1), the higher proportion of IgA1 and the occasional non-detectable levels of IgA2 in this cohort of elite swimmers may combine to increase their risk of mucosal infections. Despite a small proportion of samples having IgA2 concentrations below the assay detection limit there were no associations between IgA2 concentration or the ratios of the subclasses with infection rates.

As many of the elite swimmers in this study had been competing and training for several years, it is possible that the levels of IgA1 in the early training phase saliva collections represent a cumulative effect over the years of training of exercise-induced IgA1 suppression.

**SECOND STUDY**

The Pan Pacs Championships (swimming) were held in Sydney, Australia between 22/8/99 and 29/8/99. A further competition was held in Canberra, Australia the following week between 1/9/99 and 5/9/99. The swimmers' IgA levels and infection

data were collected during the training phase leading up to and during these competitions.

EXAMPLE 6

**Definitions**

Infection was defined as yes/no between defined time points of data collection (Table 5) after each saliva collection (Figure 3).

**TABLE 5**

| Time | Infection Data Collection Periods |
|---|---|
| 1 | 23/4/99 – 15/9/99 |
| 2 | 31/7/99 – 8/8/99 |
| 3 | 9/8/99 – 29/8/99 |
| 4 | 30/8/99 – 5/9/99 |
| 5 | 23/4/99 – 30/7/99 * |
| 6 | 31/7/99 – 30/8/99 |

* Note: Infection Logs were not recorded for 10 weeks between 15/5/99 and 30/7/99.

Saliva was collected unstimulated, non-fasting, and pre-exercise on four occasions (Table 6).

**TABLE 6**

| Training Group | Date of Collection | Subjects |
|---|---|---|
| Gold Coast | 20/4/99 | 42 |
| Brisbane | 31/7/99 | 45 |
| Melbourne | 9/8/99 | 42 |
| Sydney | 29/8/99 | 47 |

TABLE 7

## Time 1

**Comparison of Salivary Immunoglobulin Levels collected on 20/04/99 between Subjects with No Infection and Subjects with Infection during the period 23/04/99 - 15/05/99**

| | | No Infection | | | Infection | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | n | median | range | n | median | range | |
| IgA | All | 42 | 66.7 | (18-175) | 21 | 38.6 | (20-172) | 0.08 |
| | Swimmers Only | 27 | 63.5 | (18-175) | 16 | 32.7 | (20-172) | 0.03 |
| | Staff Only | 15 | 69.1 | (20-118) | 5 | 90.8 | (20-134) | 0.48 |
| | | n | median | range | n | median | range | |
| IgG | All | 42 | 8.0 | (0-44) | 21 | 7.1 | (0-37) | 0.71 |
| | Swimmers Only | 27 | 8.6 | (0-44) | 16 | 6.3 | (0-37) | 0.95 |
| | Staff Only | 15 | 7.7 | (2-30) | 5 | 17.9 | (3-24) | 0.36 |
| | | n | median | range | n | median | range | |
| IgM | All | 42 | 5.0 | (0-36) | 21 | 3.3 | (0-9) | 0.13 |
| | Swimmers Only | 27 | 5.4 | (0-36) | 16 | 3.1 | (2-9) | 0.14 |
| | Staff Only | 15 | 3.1 | (1-19) | 5 | 3.5 | (0-4) | 0.46 |
| | | n | median | range | n | median | range | |
| Albumin | All | 42 | 41.6 | (15-155) | 21 | 50.7 | (8-206) | 0.41 |
| | Swimmers Only | 27 | 45.8 | (15-155) | 16 | 47.4 | (8-180) | 0.80 |
| | Staff Only | 15 | 39.6 | (20-125) | 5 | 82.7 | (22-206) | 0.21 |

TABLE 8

Time 2

**Comparison of Salivary Immunoglobulin Levels collected on 31/07/99 between Subjects with No Infection and Subjects with infection during the period 31/07/99 - 09/08/99**

| | | No Infection | | | Infection | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | n | median | range | n | median | range | |
| IgA | All | 45 | 45.8 | (15-170) | 10 | 30.0 | (15-104) | 0.07 |
| | Swimmers Only | 31 | 47.8 | (15-134) | 8 | 30.0 | (17-104) | 0.11 |
| | Staff Only | 14 | 43.7 | (20-170) | 2 | 34.3 | (15-54) | 0.43 |
| | | n | median | range | n | median | range | |
| IgG | All | 45 | 7.5 | (0-43) | 10 | 5.4 | (3-17) | 0.39 |
| | Swimmers Only | 31 | 7.5 | (0-43) | 8 | 7.0 | (3-16) | 0.30 |
| | Staff Only | 14 | 8.2 | (3-22) | 2 | 11.0 | (5-17) | 0.63 |
| | | n | median | range | n | median | range | |
| IgM | All | 45 | 2.5 | (0-24) | 10 | 2.3 | (0-6) | 0.35 |
| | Swimmers Only | 31 | 2.2 | (0-10) | 8 | 2.7 | (0-6) | 0.70 |
| | Staff Only | 14 | 4.6 | (1-24) | 2 | 1.2 | (1-2) | 0.10 |
| | | n | median | range | n | median | range | |
| Albumin | All | 45 | 54.4 | (17-126) | 10 | 44.8 | (32-104) | 0.57 |
| | Swimmers Only | 31 | 48.8 | (17-126) | 8 | 44.8 | (34-76) | 0.83 |
| | Staff Only | 14 | 73.2 | (27-122) | 2 | 67.8 | (32-104) | 0.75 |

TABLE 9

## Time 3

**Comparison of Salivary Immunoglobulin Levels collected on 09/08/99 between Subjects with No Infection and Subjects with Infection during the period 09/08/99 - 30/08/99**

| | | No Infection | | | Infection | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | n | median | range | n | median | range | |
| IgA | All | 42 | 86.3 | (24-226) | 21 | 76.8 | (15-164) | 0.63 |
| | Swimmers Only | 27 | 78.0 | (24-164) | 11 | 71.0 | (31-164) | 0.82 |
| | Staff Only | 15 | 89.0 | (30-226) | 10 | 98.3 | (15-134) | 0.78 |
| | | n | median | range | n | median | range | |
| IgG | All | 42 | 18.1 | (1-38) | 21 | 14.2 | (5-36) | 0.37 |
| | Swimmers Only | 27 | 15.4 | (1-38) | 11 | 15.0 | (5-32) | 0.97 |
| | Staff Only | 15 | 18.8 | (5-32) | 10 | 15.0 | (7-36) | 0.07 |
| | | n | median | range | n | median | range | |
| IgM | All | 42 | 4.6 | (1-27) | 21 | 3.5 | (1-14) | 0.55 |
| | Swimmers Only | 27 | 4.2 | (1-16) | 11 | 5.4 | (2-9) | 0.65 |
| | Staff Only | 15 | 5.6 | (2-27) | 10 | 3.2 | (1-14) | 0.18 |
| | | n | median | range | n | median | range | |
| Albumin | All | 42 | 63.0 | (0-233) | 21 | 43.6 | (21-116) | 0.11 |
| | Swimmers Only | 27 | 60.8 | (0-233) | 11 | 50.5 | (26-116) | 0.68 |
| | Staff Only | 15 | 65.1 | (26-112) | 10 | 39.4 | (21-98) | 0.03 |

TABLE 10

Time 4

**Comparison of Salivary Immunoglobulin Levels collected on 29/08/99 between Subjects with No Infection and Subjects with Infection during the period 30/08/99 - 05/09/99**

| | | No Infection | | | Infection | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | n | median | range | n | median | range | |
| IgA | All | 47 | 44.1 | (20-148) | 6 | 37.3 | (28-79) | 0.48 |
| | Swimmers Only | 28 | 44.1 | (20-148) | 4 | 37.3 | (33-79) | 0.65 |
| | Staff Only | 19 | 44.1 | (26-143) | 2 | 38.9 | (28-50) | 0.55 |
| | | n | median | range | n | median | range | |
| IgG | All | 44 | 7.2 | (2-28) | 6 | 7.7 | (3-14) | 0.68 |
| | Swimmers Only | 27 | 5.9 | (2-28) | 4 | 4.6 | (3-11) | 0.38 |
| | Staff Only | 17 | 9.3 | (3-20) | 2 | 12.0 | (10-14) | 0.69 |
| | | n | median | range | n | median | range | |
| IgM | All | 47 | 3.8 | (1-22) | 6 | 3.0 | (2-6) | 0.40 |
| | Swimmers Only | 28 | 4.0 | (1-11) | 4 | 3.0 | (2-4) | 0.12 |
| | Staff Only | 19 | 3.0 | (1-22) | 2 | 4.3 | (2-6) | 0.76 |
| | | n | median | range | n | median | range | |
| Albumin | All | 47 | 39.8 | (6-153) | 6 | 29.3 | (15-80) | 0.59 |
| | Swimmers Only | 28 | 35.0 | (7-153) | 4 | 23.9 | (15-80) | 0.53 |
| | Staff Only | 19 | 47.4 | (14-140) | 2 | 53.3 | (28-78) | 0.90 |

TABLE 11

Time 5

**Comparison of Salivary Immunoglobulin Levels collected on 20/04/99 between Subjects with No Infection and Subjects with Infection during the period 23/04/99 - 30/08/99**

| | | No Infection | | | Infection | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | n | median | range | n | median | range | |
| IgA | All | 25 | 68.0 | (20-175) | 38 | 44.0 | (18-172) | **0.03** |
| | Swimmers Only | 17 | 67.7 | (20-175) | 26 | 39.1 | (18-172) | **0.02** |
| | Staff Only | 8 | 69.6 | (33-118) | 12 | 68.0 | (20-134) | 0.85 |
| | | n | median | range | n | median | range | |
| IgG | All | 25 | 9.3 | (0-44) | 38 | 7.0 | (0-37) | 0.78 |
| | Swimmers Only | 17 | 8.6 | (0-44) | 26 | 6.6 | (0-37) | 0.93 |
| | Staff Only | 8 | 11.5 | (2-27) | 12 | 7.4 | (2-30) | 0.56 |
| | | n | median | range | n | median | range | |
| IgM | All | 25 | 5.0 | (0-36) | 38 | 3.4 | (0-9) | 0.20 |
| | Swimmers Only | 17 | 5.0 | (0-36) | 26 | 3.7 | (2-9) | 0.56 |
| | Staff Only | 8 | 5.3 | (2-19) | 12 | 3.0 | (0-9) | 0.20 |
| | | n | median | range | n | median | range | |
| Albumin | All | 25 | 43.5 | (16-155) | 38 | 47.2 | (8-206) | 0.94 |
| | Swimmers Only | 17 | 37.3 | (16-155) | 26 | 47.2 | (8-180) | 0.84 |
| | Staff Only | 8 | 50.4 | (25-125) | 12 | 45.0 | (20-206) | 0.59 |

TABLE 12

## Time 6

**Comparison of Salivary Immunoglobulin Levels collected on 31/07/99 between Subjects with No Infection and Subjects with Infection during the period 31/07/99 - 30/08/99**

| | | No Infection | | | Infection | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | n | median | range | n | median | range | |
| IgA | All | 34 | 48.2 | (15-170) | 21 | 36.2 | (15-104) | 0.17 |
| | Swimmers Only | 24 | 49.0 | (15-134) | 15 | 36.2 | (17-104) | 0.23 |
| | Staff Only | 10 | 47.1 | (20-170) | 6 | 38.9 | (15-100) | 0.59 |
| | | n | median | range | n | median | range | |
| IgG | All | 34 | 7.8 | (0-43) | 21 | 5.7 | (2-21) | 0.33 |
| | Swimmers Only | 24 | 7.2 | (0-43) | 15 | 5.7 | (2-21) | 0.54 |
| | Staff Only | 10 | 10.8 | (3-22) | 6 | 5.5 | (4-17) | 0.45 |
| | | n | median | range | n | median | range | |
| IgM | All | 34 | 2.5 | (0-24) | 21 | 2.6 | (0-8) | 0.77 |
| | Swimmers Only | 24 | 2.1 | (0-10) | 15 | 2.6 | (0-8) | 0.10 |
| | Staff Only | 10 | 4.6 | (1-24) | 6 | 2.4 | (1-6) | 0.17 |
| | | n | median | range | n | median | range | |
| Albumin | All | 34 | 55.7 | (17-126) | 21 | 44.6 | (17-124) | 0.47 |
| | Swimmers Only | 24 | 45.6 | (17-126) | 15 | 45.9 | (17-124) | 0.77 |
| | Staff Only | 10 | 79.7 | (34-122) | 6 | 38.2 | (27-104) | 0.08 |

The number of subjects in the study varied at each time point (see above) due to unavailability of subjects on the day of saliva collection or exclusion of samples for various reason post analysis or Infection Logs not available.

There were no associations between salivary IgG, IgM or Albumin and infection at any of the time points studied.

There was a trend for swimmers who had an infection to have lower salivary IgA concentrations than those without infections (Tables 7 to 12) and at two time points the concentrations of pre-exercise salivary IgA were statistically significantly lower in swimmers who had an infection (Table 13).

**TABLE 13**

| Time Period | Saliva Collection | Infection Log Period | Salivary IgA No Infection (mg/L) | Salivary IgA Infection (mg/L) | p-value |
|---|---|---|---|---|---|
| 1 | 20/4/99 | 23/4/99-15/5/99 | 63.5 (18-175) | 32.7 (20-172) | 0.03 |
| 5 | 20/4/99 | 23/4/99-30/8/99 | 67.7 (20-175) | 39.1 (18-172) | 0.02 |

The results suggest that the concentration of salivary IgA in the early season training camp is the best predictor of later infection(s) during training leading up to a major competition.

The median concentration for swimmers reporting no URTIs after each time point decreased over the four month period (63.5 to 44.1 mg/L) while the concentration for swimmers with infections showed little variation (32.7 to 37.3 mg/L) (Figure 4). This suggests swimmers who are susceptible to infections may already be below a critical

threshold level of salivary IgA at the beginning of the season and have little room for further reduction with training.

The increase in salivary IgA in samples collected on 9/8/99 may be due to the fact that post this saliva collection there was an outbreak of gastrointestinal illness in the training camp, which may reflect mucosal stimulation in the elevated salivary IgA, even prior to the symptoms of gastrointestinal infection.

**THIRD STUDY**

This case study presents data on the application of salivary immunoglobulin monitoring in managing an individual elite kayaker who was prone to recurrent debilitating respiratory illness that interfered with training and preparations for competitions.

EXAMPLE 7

**Medical History**

The male elite athlete, aged 25 years, had been competing at an international level for 10 years. Five years prior to this study he contracted Epstein-Barr virus (EBV) followed by two episodes of upper respiratory tract illness (URTI). Over the proceeding years the average number of URTI had increased to 5-6 episodes per year and were associated with fatigue. A medical examination excluded all recognised clinical causes of recurrent illness and associated fatigue.

**Study Design**

Non-fasting, non-stimulated whole mixed saliva samples were collected by a standardised method (27), by the athlete before and again immediately after every exercise session over a 14-day period. An in-house ELISA measured the concentrations

of IgA, IgG and IgM and albumin concentrations were measured by rate nephelometry using a Beckman ARRAY analyser (Beckman, Brae, CA). The athlete using the PEATS program (24) an adaptation of the method described by Sharp (27), to calculate the intensity of each training session.

**Statistical Methods**

The Wilcoxon signed rank test for paired data was used to compare pre- and post-exercise session protein values. The Mann-Whitney U test was used to compare values between two selected time periods. Spearmans correlation coefficients were used to assess the association between protein values and day of training and time of day of session.

**Salivary Immunoglobulins and Albumin**

The salivary protein concentrations were all on average higher in the pre-exercise session samples compared with the post-exercise session samples (Figure 5). The median differences were statistically significant (Table 14). The salivary IgA concentrations fell significantly over the two week study (correlation coefficient (rs) = -0.55, P=0.0002) for both the pre-exercise (rs = -0.61, P=0.003) and post-exercise (rs = - 0.52, P=0.02) concentrations. There were no significant changes over time for the other salivary proteins. The magnitude of the session change (pre minus post) for salivary IgA concentrations also decreased significantly over time (rs = -0.56, P=0.01), but this was not significant for the other salivary proteins.

The magnitude of the session change for salivary IgA concentrations, but not the other proteins, was positively correlated with the intensity of the exercise session (rs = 0.53, P=0.02). The intensity of each exercise session decreased over the two-week study

period (rs = -0.45, P=0.03) and was significantly higher (P=0.05) in the first four days (median score = 165, range = 80-217) compared to the subsequent ten days of training (median score = 110, range = 56-185). The concentrations of IgA in both the pre-exercise and post-exercise session samples were significantly higher (P=0.009) for the first four days (median = 164 mg/L, range = 28 – 228 mg/L) compared to the last ten days (median = 51 mg/L, range = 30 – 72 mg/L). The magnitude of the session change in concentrations for salivary IgA were significantly greater (P=0.02) in the first four days (median = 115 mg/L, range = -44 – 137) compared to the next ten days (median = 20 mg/L, range = -30 - 47 mg/L), being almost six times greater. This was primarily due to the drop in pre-exercise session salivary IgA concentration during the fourth day of training and lack of subsequent recovery (Figure 5).

Multiple exercise sessions were undertaken during some of the training days. The average percent recovery of the initial IgA value for the day during the first three days was 74% (Figure 6). On day four the recovery for the second exercise session was only 20%. Once the initial IgA concentrations fell below 50 mg/L (days 8-13) the IgA concentration showed little variation with additional training during the day (Figures 5 & 6).

**Discussion**

An adequate level of immune protection at mucosal surfaces is important for resistance against infection (23). Low levels of salivary IgA have been associated with a risk of URTI in cohorts of elite athletes (16,26,18,28). Monitoring every episode of training in this case study allowed the identification of the exact exercise session that resulted in a significant suppression of the salivary IgA concentration without

subsequent recovery (day 4). An important finding was the association between the early high intensity exercise with the magnitude of subsequent mucosal immune suppression, reflected by lower levels of salivary IgA after the intense exercise period.

This study highlights the benefit to the athlete and the coach if results of the salivary IgA concentration assays are available rapidly. Without the benefit of the knowledge of the mucosal immune suppression the athlete continued intensive training until restricted by a further episode of respiratory illness. In individual athletes who are prone to infection, or whose performance has declined, extensive monitoring of the athlete's training program may assist with identifying training factors that contribute to the mucosal immune suppression and potential risk of infection.

**TABLE 14**

The median and range, and the median session difference for the pre-training and post-training salivary immunoglobulins and albumin concentrations (mg/L) over the two week study.

| Salivary Protein | Pre-training | | Post-training | | Session Difference | | Significance |
|---|---|---|---|---|---|---|---|
| | Median (mg/L) | Range (mg/L) | Median (mg/L) | Range (mg/L) | Median Difference | | p-value |
| | | | | | (mg/L) | (95% CI) | |
| IgA | 54.0 | 28 - 228 | 39.5 | 13 - 95 | 29 | 13.6 - 53.4 | 0.006 |
| IgG | 6.0 | 1 - 17 | 6.0 | 1 - 14 | 3 | -0.6 - 6.3 | 0.04 |
| IgM | 4.0 | 1 - 22 | 1.5 | 1 - 9 | 2 | 0.7 - 4.0 | 0.01 |
| Albumin | 56.0 | 17 - 184 | 36.0 | 20 - 89 | 15 | 6.4 - 34.3 | 0.003 |

Although the invention has been described with reference to specific examples it will be appreciated by those skilled in the art that the invention may be embodied in many other forms.

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**CLAIMS:**

1. A method for assessing potential susceptibility to infection in a subject exposed to a stressor or stressors including:

(a) determination of the subject's immunoglobulin A (IgA) level in a training phase; and

(b) prediction of the subject's susceptibility to infection by comparison of the training phase IgA level with a predetermined threshold value.

2. A method for assessing potential susceptibility to infection in a subject exposed to a stressor or stressors including:

(a) determination of the subject's immunoglobulin A1 (IgA1) level; and

(b) prediction of the subject's susceptibility to infection by comparison of the IgA1 level with a predetermined threshold value.

3. A method according to claim 1 or claim 2 wherein the immunoglobulin level is determined in early training phase.

4. A method according to any one of claims 1 to 3 wherein the infection is a mucosal infection.

5. A method according to claim 4 wherein the infection is a respiratory infection.

6. A method according to any one of claims 1 to 5 wherein the threshold value is a normal population threshold value.

7. A method according to any one of claims 1 to 5 wherein the threshold value is an internal personal threshold value.

8. A method according to any one of claims 1 to 7 wherein the immunoglobulin is secretory immunoglobulin.

9. A method according to claim 8 wherein the secretory immunoglobulin is salivary immunoglobulin.

10. A method according to claim 9 wherein the salivary immunoglobulin is from a sample of whole unstimulated saliva.

11. A method according to claim 10 wherein the subject is not fasting when the saliva is collected.

12. A method according to any one of claims 1 to 11 wherein the subject is an athlete.

13. A method according to claim 12 wherein the athlete is an elite swimmer.

14. A method according to any one of claims 1 to 13 wherein the stressor is known to influence the efficacy of the immune system.

15. A method according to any one of claims 1 to 13 wherein the subject is exposed to a physical and/or psychological stressor.

16. A method according to claim 15 wherein the physical and/or psychological stressor is long-term physical training or overtraining.

17. A method according to any one of claims 1 to 16 wherein the immunoglobulin level is determined by radial immunodiffusion.

18. A method according to any one of claims 1 to 16 wherein the immunoglobulin level is determined by ELISA.

19. A method according to any one of claims 1 to 18 wherein the immunoglobulin level is determined by a rapid near-subject assay.

20. A method according to any one of claims 1 to 19 wherein the saliva is analysed for the immunoglobulin content *in situ* by contacting an assay device or system with the saliva of a subject.

21. A method according to any one of claims 1 to 20 wherein the assay is a self-test performed by the subject.

22. A method for monitoring a subject's progress following intervention therapy including:

(a) determination of the subject's IgA1 level; and

(b) monitoring the subject's progress by comparison of said IgA1 level with a predetermined threshold value.

23. A method according to claim 22 wherein the IgA1 level is assessed from a sample of the subject's saliva.

24. A method for assessing the impact of a stressor or stressors on a subject's performance and/or fatigue levels, as defined herein, including:

(a) determination of the subject's salivary IgA1 level; and

(b) assessing the impact of the stressor or stressors on the subject's performance and/or fatigue levels by comparison of said IgA1 level with a predetermined threshold value.

25. A method according to claim 24 wherein the subject is an athlete.

26. A method according to claim 25 wherein the athlete is an elite swimmer.

27. A method for assessing potential susceptibility to respiratory infection in an elite swimmer, including:

(a) determining the swimmer's salivary immunoglobulin A (IgA) level in early training phase; and

(b) predicting the swimmer's potential susceptibility to respiratory infection by comparison of the early training phase IgA level with a predetermined threshold value.

28. A method for assessing potential susceptibility to respiratory infection in an elite swimmer, including:

(a) determining the swimmer's salivary immunoglobulin A1 (IgA1) level in early training phase; and

(b) predicting the swimmer's potential susceptibility to respiratory infection by comparison of the early training phase IgA1 level with a predetermined threshold value.

29. A method according to claim 28 wherein the threshold value is a normal population threshold value.

30. A method according to claim 28 wherein the threshold value is an internal personal threshold value.

31. A method for assessing a subject's potential susceptibility to infection including:

(a) determining the subject's immunoglobulin A (IgA) level after exposure to a stressor or stressors;

(b) allowing a recovery period as herein defined;

(c) determining the subject's IgA level after the recovery period;

(d) predicting the subject's potential susceptibility to infection by comparison of the IgA level at step (a) above with the IgA level at step (c) above.

32. A method for assessing a subject's potential susceptibility to infection including:

(a) determining the subject's immunoglobulin A1 (IgA1) level after exposure to a stressor or stressors;

(b) allowing a recovery period as herein defined;

(c) determining the subject's IgA1 level after the recovery period;

(d) predicting the subject's potential susceptibility to infection by comparison of the IgA1 level at step (a) above with the IgA1 level at step (c) above.

33. A method according to claim 31 or 32 wherein the infection is a mucosal infection.

34. A method according to claim 33 wherein the infection is a respiratory infection.

35. A method according to any one of claims 31 to 34 wherein the immunoglobulin is secretory immunoglobulin.

36. A method according to claim 35 wherein the secretory immunoglobulin is salivary immunoglobulin.

37. A method according to claim 36 wherein the salivary immunoglobulin is from a sample of whole unstimulated saliva.

38. A method according to claim 37 wherein the subject is not fasting when the saliva is collected.

39. A method according to any one of claims 31 to 38 wherein the subject is an athlete.

40. A method according to claim 39 wherein the athlete is an elite swimmer.

41. A method according to any one of claims 31 to 40 wherein the stressor is known to influence the efficacy of the immune system.

42. A method according to any one of claims 31 to 40 wherein the subject is exposed to a physical and/or psychological stressor.

43. A method according to claim 42 wherein the physical and/or psychological stressor is long-term physical training or overtraining.

44. A method according to any one of claims 31 to 43 wherein the immunoglobulin level is determined by radial immunodiffusion.

45. A method according to any one of claims 31 to 43 wherein the immunoglobulin level is determined by ELISA.

46. A method according to any one of claims 31 to 45 wherein the immunoglobulin level is determined by a rapid near-subject assay.

47. A method according to any one of claims 31 to 46 wherein the saliva is analysed for the immunoglobulin content *in situ* by contacting an assay device or system with the saliva of a subject.

48. A method according to any one of claims 31 to 47 wherein the assay is a self-test performed by the subject.

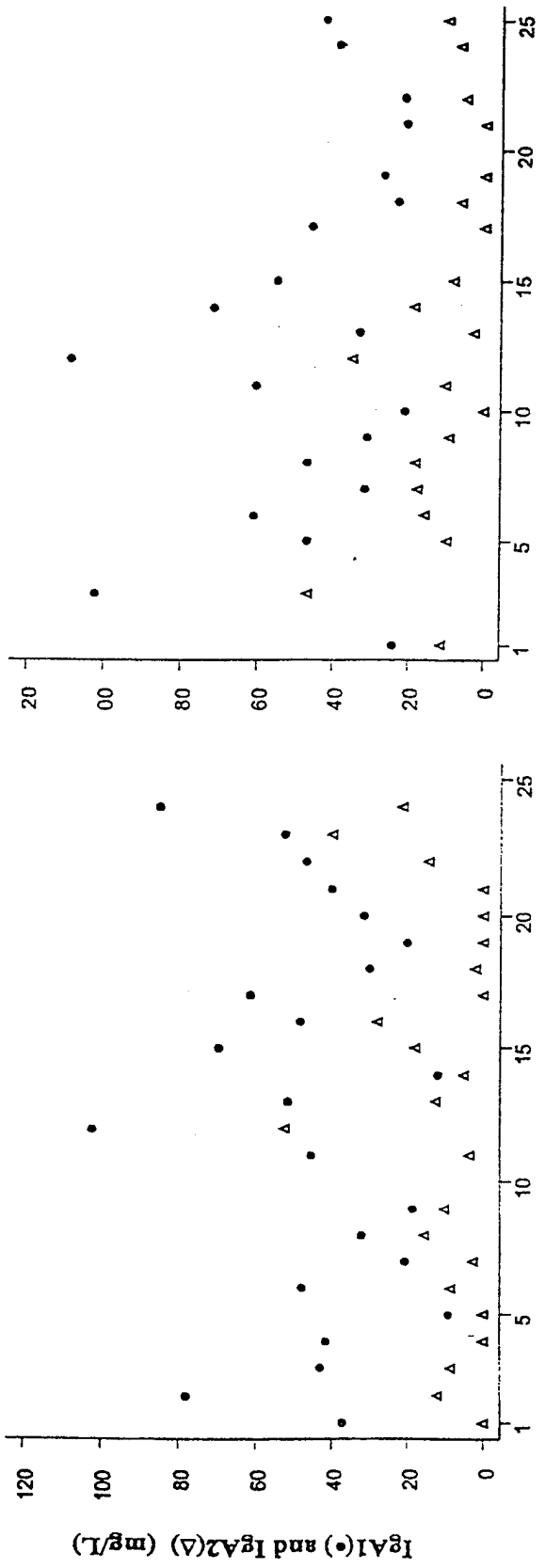

IgA1(•) and IgA2(Δ) (mg/L)
Early Training Phase Sample
Late Training Phase Sample
Individual Athletes
Individual Athletes

FIGURE 1

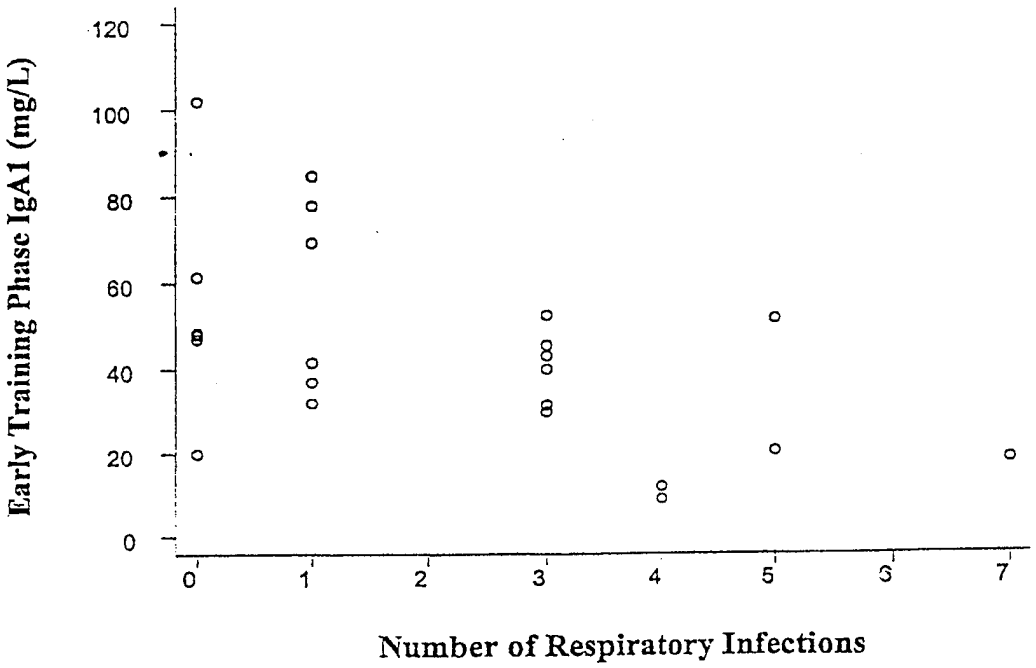
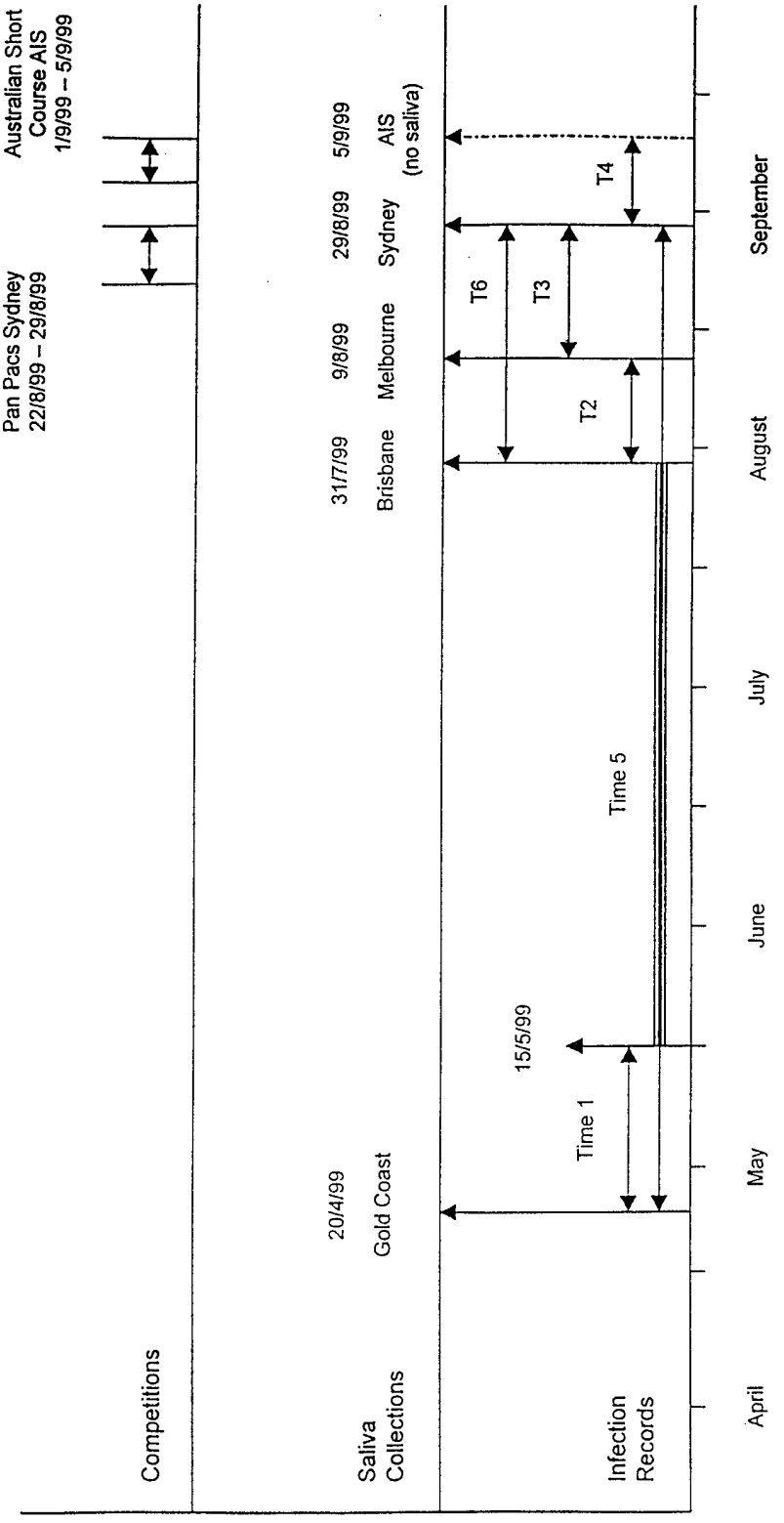

Early Training Phase IgA1 (mg/L)
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
Number of Respiratory Infections

FIGURE 2

Pan Pacs – Salivary IgA/Infection Study
Figure 3 – Infection Data Time Points



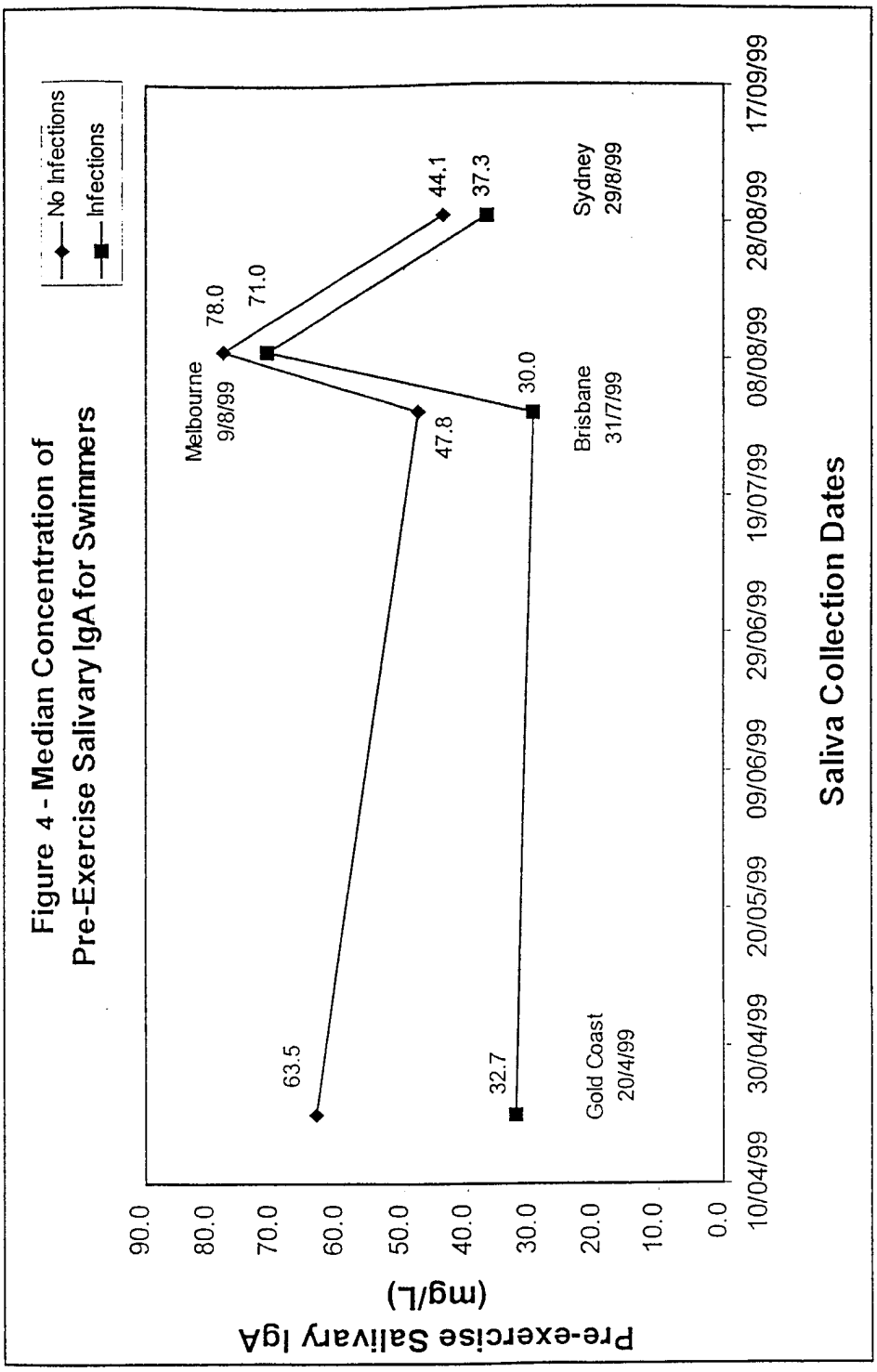
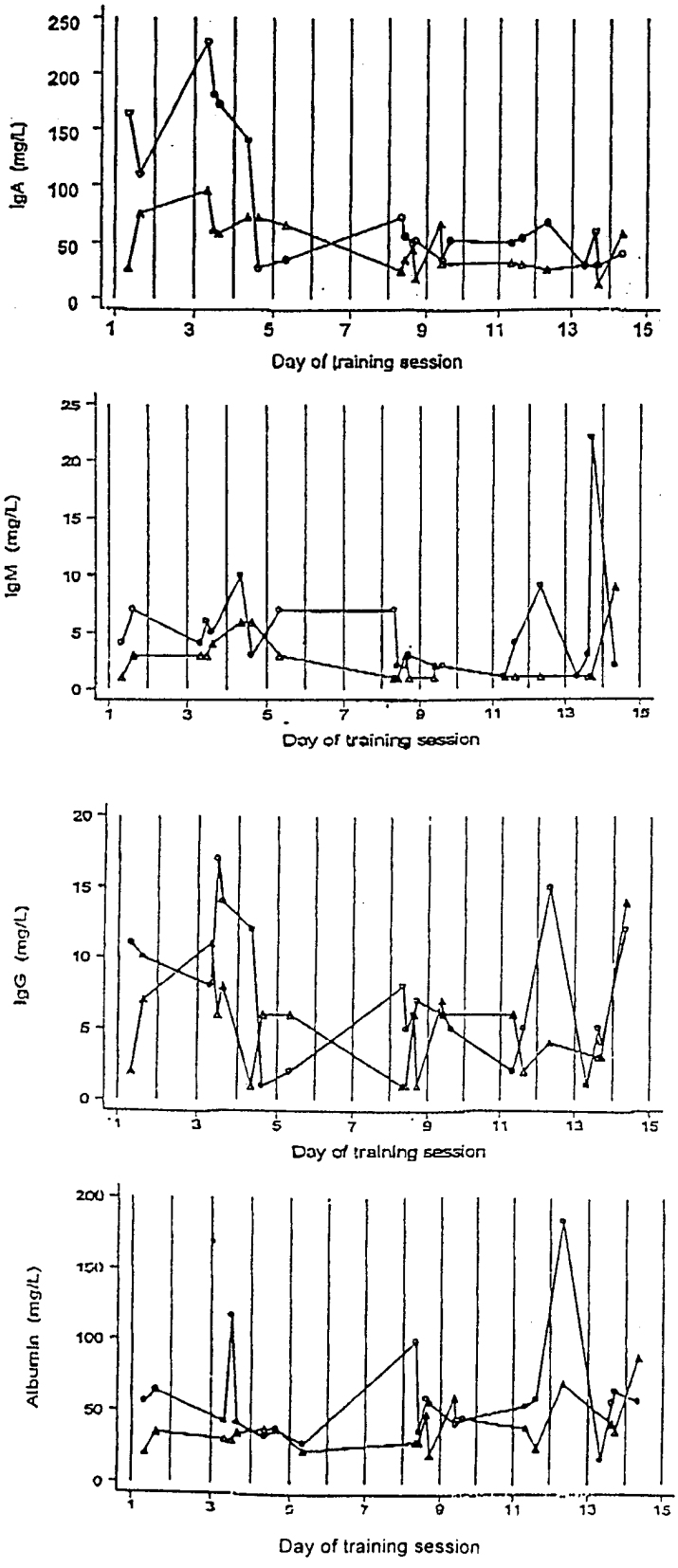


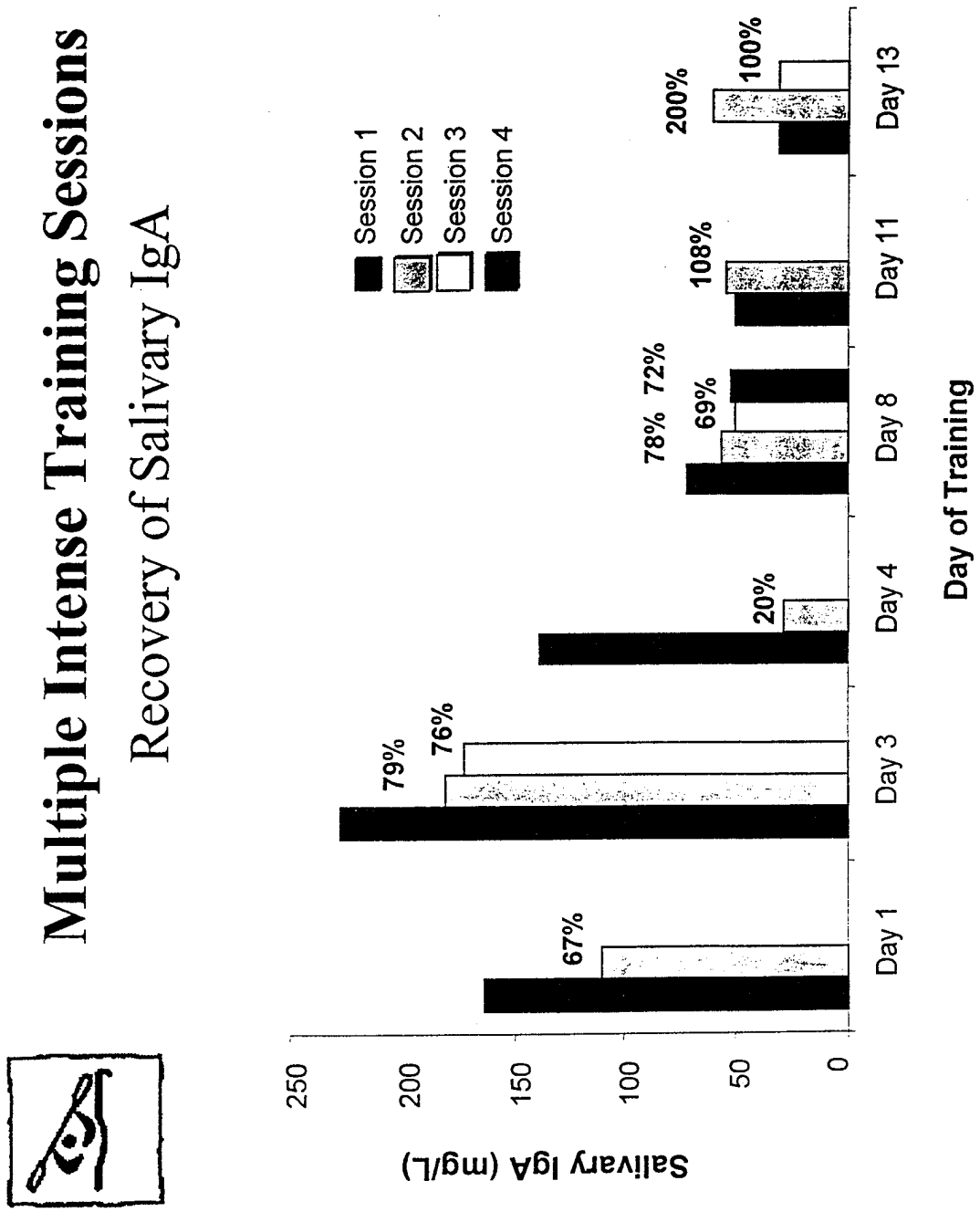
Figure 4 - Median Concentration of Pre-Exercise Salivary IgA for Swimmers
No Infections
Infections
Pre-exercise Salivary IgA (mg/L)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
10/04/99
30/04/99
20/05/99
09/06/99
29/06/99
19/07/99
08/08/99
28/08/99
17/09/99
Saliva Collection Dates
Melbourne 9/8/99
Brisbane 31/7/99
Gold Coast 20/4/99
Sydney 29/8/99
78.0
71.0
47.8
63.5
30.0
32.7
44.1
37.3

FIGURE 5




Pre-exercise (o--o)
and
Post-exercise (Δ--Δ)

FIGURE 6


Multiple Intense Training Sessions
Recovery of Salivary IgA
Session 1
Session 2
Session 3
Session 4
Salivary IgA (mg/L)
250
200
150
100
50
0
Day 1
Day 3
Day 4
Day 8
Day 11
Day 13
Day of Training
67%
79%
76%
20%
78%
72%
69%
108%
200%
100%

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/AU 00/00085**

**A. CLASSIFICATION OF SUBJECT MATTER**

Int Cl[7]: G01N 33/68

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPIDS, JAPIO, MEDLINE, CA; KEYWORDS IGA, IGA1, SIGA, EXERCISE, ATHLETICS, SWIMMING, RESPIRATORY, INFECTION, SALIVA

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | GLEESON M, et al, "Salivary IgA subclasses and infection risk in elite swimmers", Immunol. Cell Biol, Aug 1999, Vol 77, No. 4, p351-5<br>Whole document | 1-48 |
| X | GLEESON M, et al, "Salivary IgA levels and infection risk in elite swimmers" Med. Sci. Sports Exerc. Jan 1999; Vol 31, No.1, p67-73<br>Whole document | 1, 3-21, 27, 31,33-48 |
| X | PYNE DB et al, "Effect of intensive training on immunity in athletes" Int. J. Sports Med., July 1998, Vol 19 suppl 3 s183-91<br>S185-186 | 1, 3-21, 27, 31,33-48 |

[X] Further documents are listed in the continuation of Box C

[ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 29 February 2000 | 14 MAR 2000 |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | Authorized officer<br>**ROSS OSBORNE**<br>Telephone No.: (02) 6283 2404 |

| INTERNATIONAL SEARCH REPORT | International application No. PCT/AU 00/00085 |
|---|---|

**C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | MACKINNON LT, "Mucosal (secretory) immune responses to exercise of varying intensity and during overtraining", Int. J. Sports Med., October, Vol 15, 1994, Suppl 3, S179-83<br>See whole document | 1, 3-21, 27, 31,33-48 |
| Y | THARP G D et al, "Reduction of saliva immunoglobulin levels by swim training", Eur. J. App Physiol. 1990, Vol 60 No.1 p61-62,<br>See whole document | 1, 3-21, 27, 31, 33-48 |
| P, A | WARNER RH et al "Salivary SIgA and SIgA 1 in coeliac disease, inflammatory bowel disease and controls", Ir. J. Med. Sci., 1999, Jan Mar, Vol 168, No.1, p33-5 | |

| | | | |
|---|---|---|---|
| 专利名称(译) | 与剧烈运动或其他压力相关的感染易感性 | | |
| 公开(公告)号 | EP1181557A1 | 公开(公告)日 | 2002-02-27 |
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| 发明人 | CLANCY, ROBERT, LLEWELLYN<br>GLEESON, MAREE | | |
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| 优先权 | 1999PP8603 1999-02-10 AU | | |
| 其他公开文献 | EP1181557B1<br>EP1181557A4 | | |
| 外部链接 | Espacenet | | |


摘要(译)

本发明涉及用于确定暴露于应激物的受试者中感染的易感性的方法。特别地，本发明涉及通过监测IgA和IgA1水平评估受试者易感染风险的方法。