Salivary Immunoglobulin-A as a Marker of Stress During Strenuous Physical Training

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Background: Immunoglobulin-A in saliva (SIgA) has been proven to be decreased during periods of stress, a change that also correlates with increased disease risk. ***Hypothesis:*** Concentration of SIgA is negatively associated with dietary deprivation, negative moods, and anxiety. ***Methods:*** SIgA was evaluated as a marker of the severity of stress during a 19-day Royal Australian Air Force (RAAF) survival course, during which students experienced hunger, thirst, boredom, loneliness, and extreme heat and cold combined with demanding physical effort. There were 27 men and 2 women who participated. Students kept daily food diaries, from which daily intakes of energy and macronutrients were calculated. Saliva samples were collected on 9 d for the measurement of the ratio of SIgA to albumin (Alb). Students completed a health checklist and the State Anxiety Inventory on the same 9 d and the Profile of Mood States on 3 of the days. ***Results:*** Dietary restriction, consumption of alcohol, body mass loss, occurrence of upper respiratory tract infection, and negative emotions were negatively associated with SIgA:Alb. ***Conclusion:*** SIgA:Alb is a useful marker of the severity of stresses encountered during stressful training.

Keywords: salivary immunoglobulin-A, dietary restriction, psychological stress.

MAINTAINING THE HEALTH of defense personnel is integral to ensuring a high level of military performance. Physical activity, exposure to the elements, periods of inadequate nutrition, and psychological challenges are all factors that could have an impact on the health of personnel. A simple diagnostic test, which indicates an increasing vulnerability to illness, could be used to assess the stress of training programs on military personnel. With the assistance of a simple stress marker, practices such as altered work-rest cycles, improved diet, and psychological techniques could be used as tools to minimize the impact of training on the health of military personnel.

Secretory immunoglobulin-A (Ig-A) is found at the mucosal membranes and represents the first line of defense against viruses and bacteria. Because most infectious agents enter the host via the mucosal membranes, decreased concentration of secretory IgA represents a reduced level of protection for the body and an increased risk of infection. Saliva, tears, nasal fluids, bronchial, intestinal, and genitourinary secretions contain immunoglobulins, predominantly secretory IgA (4). Secretory IgA protects the mucosal surfaces through several mechanisms, which result in the inability of pathogens to attach to mucosal surfaces, thereby preventing entry into the body, limiting viral replication, or aiding in elimination (7). The levels of secretory IgA are correlated with resistance to certain viruses responsible for upper respiratory tract infections (URTI) (17).

In a study of U.S. army recruits performing an 8-wk physical training program about one-third were classified as overtrained (3). Overtraining is typified by a decrease in performance, but other symptoms can include fatigue; mood disturbances including apathy, irritability, anger, and depression; frequent URTI; injury; muscle soreness; and joint pain (17).

Athletes undergoing high intensity training have a high incidence of URTI (5,21). Concentrations of salivary immunoglobulin-A (SIgA) have been suppressed after intense exercise in elite swimmers (8), in moderately active runners (16), cross country skiers (13), distance runners (18), cyclists (23), and kayakers (14).

There is also indication that exposure to psychosocial stressors and negative moods leads to immune suppression (11,15). A negative relationship has been found between the number of daily hassles and SIgA levels (6). The secretion rate of SIgA was lower in nurses who reported that they were "frequently anxious" (9) and it was also lower in students during high stress periods (10). Positive associations have been found between SIgA concentration and positive mood states (12,22).

Due to the ease of saliva collection, measurement in the field may be feasible if a robust and cheap method of SIgA analysis can be developed. However, it would first be necessary to verify that SIgA is a sensitive marker of the overtraining problems that can occur during military training. The RAAF survival school course presented a good opportunity to evaluate SIgA as a marker of stress in physically, mentally, nutritionally, and emotionally stressed adults.

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METHODS

Participants and Survival Course

This study was conducted during the November 1999 RAAF survival course. Of the 29 students who agreed to participate, only 2 were women. Five of the men were cigarette smokers. The mean age of the group was 26 yr (range 18–39). Most students were in the normal body mass range with a mean body mass index (BMI) of 24 (range 20–31). The Australian Defense Human Research Ethics Committee approved the experimental procedures. Written consent was obtained from each participant following an explanation of the details of the study.

On arrival the students were required to complete physically challenging activities to determine if they possessed the minimum physical and mental fitness needed to attempt the course. This initial (baseline) phase was conducted over 3 d (days 0 to 2). The students were then required to demonstrate survival skills in three different environments, a sea phase (days 3 to 4.5), an arid phase (days 7 to 9.5), and a jungle phase (days 13 to 18).

The final jungle phase, which involved an escape and evasion exercise, was the most demanding. Students evaded an enemy for 2 d, during which time they covered up to 25 km over rough terrain. They then employed survival skills for another 4 d in the jungle, the last 2 of which were spent in a solitary situation. They were provided with less than 2.0 MJ per day in emergency rations, so they searched for native foods and water sources.

While in barracks (between phases) students were fed in the dining hall and had access to a bar which served alcoholic drinks in the evening. They were required to complete at least two bouts of strenuous activity each day, such as a 350 m climb in 2 km, over rocky terrain. These activities were time-limited and those who failed to meet the requirement were dropped from the course.

Methods

Height (m) and body mass (kg) were measured at baseline (day 2) and body mass was measured again on days 13 (pre-jungle phase) and 18 (post-jungle phase).

Students maintained a food intake diary for the duration of the course. This data allowed the daily intake of energy and macronutrients (carbohydrate, protein, fat, and alcohol) to be calculated by use of the Foodworks software (Version 2.05, 1999, Xyris Software, QLD, Australia), which used the NUTTAB 95 food composition database. Foodworks software was also used to estimate each participant's daily dietary requirements.

Three saliva samples were donated during the baseline phase on day zero of the course. Additional saliva samples were collected immediately before and after each of the survival phases and twice during the final jungle phase. Participants were required to have a clean, freshly rinsed mouth and to retain the cotton swab in their mouth without chewing until they had to swallow. The student then spat the cotton swab into a Salivette tube (Sarstedt, Nümbrecht, Germany). Samples were transported cold to the barracks where they were stored frozen. Alb and SIgA were measured by nephelometric assay (Behring BNA II, Marburg, Germany) using manufacturer-supplied reagents (antisera to Human IgA α chain and human albumin, Behring Diagnostics, Marburg, Germany). The results were presented as the ratio of SIgA ($mg \cdot L^{-1}$) to Alb ($mg \cdot L^{-1}$).

Students completed a simple health checklist when they collected each saliva sample. This included the following health problems: cough, cold, runny nose, sore throat (suspected URTI), skin complaints, rashes, sores, dehydration, insect bites, headaches, allergic reactions, stomach upset, nausea, vomiting, exhaustion, fracture, dislocation, muscle strain, sunburn, and other physical symptoms. Each symptom was scored as present or absent at each time point to give a physical condition score of between 0 and 12.

The Profile of Mood States questionnaire (POMS; McNair, Lorr, and Droppleman, eds.) was used to assess changes in mood and the State Anxiety Inventory, Form Y (STAI, Speilberger CD, Mind Garden Inc., CA) was used to assess changes in anxiety during the survival course. The STAI was administered at the same time as the health checklist and the POMS was administered (at the same time each day) on 3 d (baseline, pre-jungle, and post-jungle). The STAI results were presented as a single (weighted) score and the POMS was scored for each of the factors: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, confusion-bewilderment, and total mood disturbance.

Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences, version 9.0, 1999, SPSS Inc., Chicago, IL). Descriptive statistics were obtained to establish a measure of central tendency and are presented as means, standard deviations, and range. Data were checked for outliers and non-homogeneity of the population by use of pair-wise scatter plots, box plots, and Q-Q plots. Significance was accepted at $p < 0.05$. Multiple linear regression analyses and logistic regression were used to assess associations between variables. Repeated measures analysis of variance was used to determine significant change in responses for tests with serial measurements. All within-subject F-tests were based on the Huynh-Feldt corrected p-values. Only when these tests provided evidence of difference were pair-wise comparisons employed. Within-subject comparisons were obtained as pre-planned contrasts specified in the repeated measures analysis of variance performed by SPSS.

RESULTS

Mean body mass progressively decreased during the survival course ($F = 452$, $p < 0.01$). However, most of the loss occurred during the jungle phase ($p < 0.01$). All students lost body mass during this phase of the survival course (mean loss 6.1 ± 1.3 kg). The loss over the course was 6.5 ± 1.4 kg or a mean of 9% of initial body mass (range 3.4–10 kg, 6% to 12.5%).

The daily dietary requirements in barracks were determined as: energy 13.6 ± 1.1 MJ, carbohydrate 450 ± 40 g, protein 90 ± 7 g, and fat 110 ± 9 g. Daily dietary

TABLE I. MEAN DAILY INTAKE OF ENERGY AND MACRONUTRIENTS BY PARTICIPANTS FOR EACH OF THE SURVIVAL SCHOOL PHASES.

	Baseline in Barracks	Sea Phase	Post-Sea in Barracks	Arid Phase	Post-Arid in Barracks	Jungle Phase
Energy ($MJ \cdot d^{-1}$)	12.5 ± 3.5	2.2 ± 1.2	11.8 ± 5.4	1.9 ± 0.8	12 ± 4.1	1.4 ± 0.4
Protein ($g \cdot d^{-1}$)	140 ± 30	20 ± 10	110 ± 60	30 ± 8	120 ± 40	10 ± 4
Carbohydrate ($g \cdot d^{-1}$)	360 ± 130	70 ± 50	310 ± 170	50 ± 30	330 ± 110	44 ± 10
Fat ($g \cdot d^{-1}$)	110 ± 30	20 ± 8	100 ± 50	20 ± 7	100 ± 40	10 ± 3
Alcohol ($g \cdot d^{-1}$)	5 ± 12		34 ± 43		27 ± 27	

requirements during survival phases were determined as: energy 15.8 ± 1.5 MJ, carbohydrate 550 ± 50 g, protein 90 ± 7 g, and fat 130 ± 12 g. **Table I** presents the mean daily intakes of macronutrients and energy.

Salivary IgA:Alb levels fluctuated during the survival course (**Fig. 1**, F = 13.29, $p < 0.01$). Mean levels recorded at the end of each in-barracks phase were stable and on average not different than the baseline level. Levels dropped significantly after the arid ($p < 0.01$) and jungle ($p < 0.01$) phases. The jungle phase had the greatest impact on SIgA:Alb with a significant drop recorded on the fourth day of this phase and the lowest mean level of the course recorded after completion of this phase ($p < 0.01$).

There was evidence for fluctuations in anxiety (mean STAI scores) during the course (**Fig. 2**, F = 10.71, $p < 0.01$). The greatest periods of anxiety were at baseline ($p < 0.01$) and early in the jungle phase ($p < 0.01$). Mild increases in anxiety due to the other two phases were not significant.

Changes in the mood factors tension-anxiety (F = 10.20, $p < 0.01$), vigor-activity (F = 5.18, $p < 0.01$), fatigue-inertia (F = 52.84, $p < 0.01$), and total mood disturbance (F = 3.93, p = 0.04) were significant. Mood changed little during the early phases of the course. Both vigor-activity and tension-anxiety tended to decrease from baseline to pre-jungle and decreased significantly on conclusion of the jungle phase (p = 0.01, $p < 0.01$, respectively). Similarly for the factors fatigue-inertia and total mood disturbance the scores tended to increase across the early part of the course, but only increased significantly during the jungle phase (p = 0.00, p = 0.03, respectively).

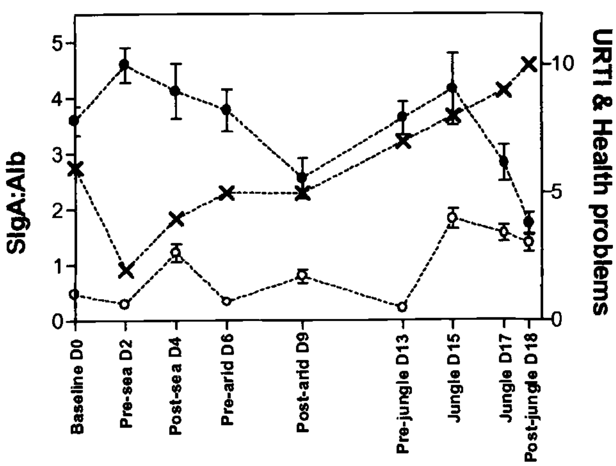

Fig. 1. Mean health problems scores (○) and total frequency of URTI (X) in relation to mean SIgA:Alb (●). Error bars indicate SEM.

Health problems progressively increased during the survival course (health problems scores, F = 35.45, $p < 0.01$) with a significant increase during each survival phase ($p < 0.01$). As expected health problems peaked during the jungle phase ($p < 0.01$). Fig. 1 shows the mean health problem scores recorded during each phase of the survival course in relation to mean SIgA:Alb and recorded frequency of URTIs.

Dietary intake, loss of body mass, and fatigue were associated with SIgA:Alb levels (**Table II**). There was some weak evidence that the negative moods, depression-dejection (r = 0.43, F = 5.98, p = 0.02), and anger-hostility (r = 0.55, F = 11.02, $p < 0.01$) measured on the morning of the jungle phase and the increase in anxiety (STAI score, r = 0.40, F = 5.18, p = 0.03) measured early during the jungle phase were associated with the subsequent drop in SIgA:Alb.

There is some evidence that alcohol consumption during in-barracks phases had a negative association with SIgA:Alb. For example, average alcohol consumption while in-barracks was negatively associated with SIgA:Alb measured at the end of these phases (Table II). When moderate drinkers (≤40 g alcohol for men and ≤20 g alcohol for women) were compared with heavy drinkers (one-way analysis of variance) there was strong evidence that drinking prior to the arid survival phase had an effect—there was a decrease in SIgA:Alb for the heavy drinkers and an increase for the moderate drinkers ($p < 0.01$), but drinking before the jungle phase had no effect.

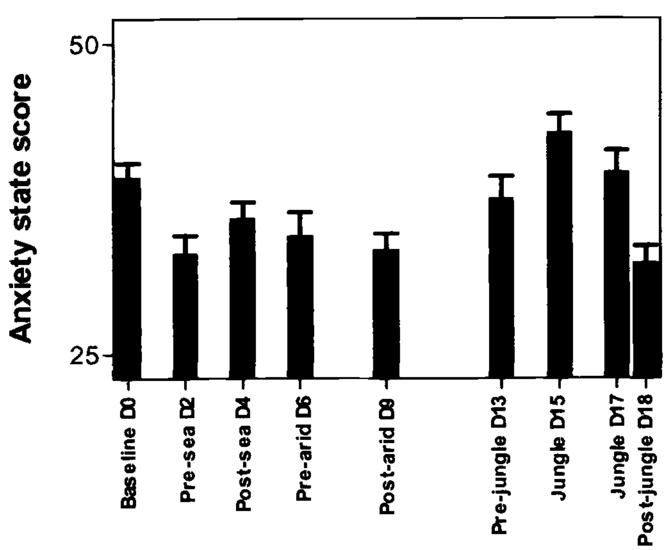

Fig. 2. Mean and SEM for STAI scores recorded during each phase of the survival school course.

TABLE II. PEARSON CORRELATION COEFFICIENTS FOR SIGNIFICANT CORRELATIONS BETWEEN DIETARY INTAKE, CHANGES IN BODY MASS, PSYCHOLOGICAL FACTORS AND SALIVARY IMMUNOGLOBULIN-A.

	SIgA:Alb*	P
Energy‡ (MJ · d^{-1})	0.692	<0.01
Carbohydrate (g · d^{-1})	0.703	<0.01
Protein (g · d^{-1})	0.613	<0.01
Fat (g · d^{-1})	0.649	<0.01
Alcohol/kg body mass (g · d^{-1})	−0.317	<0.01
% BMI	0.642	<0.01
Fatigue-inertia	−0.324	0.01

* SIgA:Alb is the ratio of salivary immunoglobulin-A (mg · L^{-1}) to albumin (mg · L^{-1}).
‡ Macronutrient and alcohol consumption was averaged over each phase and compared with SIgA:Alb recorded at the end of the phase. %BMI refers to the change in body mass recorded for the period preceding the SIgA:Alb measurement.

Multinomial logistic regression analysis revealed a negative association between SIgA:Alb and health problem score (χ^2 = 218, df = 180, p = 0.03). When the frequency of the various health complaints during each survival phase were compared with the average SIgA: Alb recorded by all students during each survival phase, URTI was found to be negatively correlated with SIgA:Alb (r = −0.62, p = 0.04).

Body mass was found to be negatively associated with the health problems score (r = 0.40, p = 0.02). Health problems were also associated with the STAI score (r = 0.39, p = 0.02) and the mood factors, tension-anxiety (r = 0.43, p = 0.01), depression-dejection (r = 0.44, p = 0.01), anger-hostility (r = 0.46, p = 0.01), fatigue-inertia (r = 0.61, p < 0.01), confusion-bewilderment (r = 0.48, p < 0.01), and total mood disturbance (r = 0.49, p < 0.01).

There was no evidence that cigarette smokers differed with non-smokers in any of the psychological, dietary, or immunological measures recorded in this study.

DISCUSSION

On arrival at the survival school, the students appeared well nourished and physically fit. On the first day of the course students may have been feeling anxious about the course as evidenced by their scores for the POMS mood factor tension-anxiety and anxiety as measured by the STAI (21). The first few days of the course were spent in barracks and showed positive health effects for the students. For example the students' mean SIgA:Alb improved during this time (Fig. 1), the number of URTIs reported decreased (Fig. 1), and the level of anxiety decreased as students became involved in the course (Fig. 2).

While in-barracks, students could self-select from meals served in the RAAF mess. It is noteworthy that the students' choice of foods provided more than sufficient protein and fat, but generally inadequate carbohydrate and energy. On the days between the arid and jungle phases, more than half the students drank greater than the four standard alcoholic drinks per day advised by the National Health and Medical Research Council as the maximum safe level (19).

Levels of high physical activity and malnutrition were experienced during the survival phases of the course. The survival phases were characterized by increased reporting of health problems, particularly dehydration, insect bites, and exhaustion, and decreased SIgA:Alb. Twelve students experienced nausea during the sea-phase. The jungle phase was the most emotionally and physically demanding survival phase as evidenced by the largest drops in SIgA:Alb, body mass, and vigor and increased anxiety, fatigue, and mood disturbance. Despite the less-than-optimal food consumption while in barracks, SIgA:Alb levels appeared to recover between survival phases.

Many factors may have contributed to the changes in students' SIgA:Alb during the survival course. Those which were shown to have a negative relationship with SIgA:Alb included inadequate intake of energy and macronutrients, consumption of alcohol, loss of body mass, and the negative emotions of anger, fatigue, depression, and anxiety. Carbohydrate might have been the most limiting macronutrient in terms of mucosal immune function.

The evidence for the effect of alcohol on SIgA:Alb was equivocal, because drinking prior to the arid phase had a suppressive effect while drinking prior to the jungle phase had no effect. It should be noted that participants were not randomly allocated to the low and high drinking groups and that the composition of the two groups differed with each drinking occasion. Hence, there may be other factors which distinguish the groups and are responsible for the observed group differences.

There is little published literature describing the effects of smoking and alcohol on SIgA levels because most studies have precluded these variables in their design. One study which involved 114 female nurses found that age, smoking habits, and alcohol consumption were not related to SIgA secretion rate (9). Studies of soldiers under field conditions has provided some weak evidence for the negative effect of cigarette smoking on SIgA:Alb (1,2). These studies have also indicated that heavy smokers are likely to under-consume combat rations with a resultant loss of body mass which is greater than for non-smokers. Further investigation of the interactions between smoking, alcohol consumption, and mucosal immunity is warranted.

Decreased SIgA:Alb was also associated with increased reporting of URTI and with total health problems. The incidence of URTI steadily increased over the course. Factors that may possibly influence SIgA:Alb levels but were not examined during this study included sleep quality and heat strain.

It is likely that a lag exists between an acute change in mood and the immune function response. The negative effect of anger, depression, and anxiety on the subsequent change in SIgA:Alb recorded during the jungle survival phase lends support to this idea. Furthermore, the SIgA response to chronic hassles has been shown to be delayed for several weeks (6). Although this study indicated an interaction between the negative emotions and declining SIgA:Alb levels, further research is required to determine the interaction between mood states and immune function. In particular, more sensitive measures of fatigue, including sleep quality, need to be used in future studies.

It was not practical to monitor students' mood and state of anxiety closely during the survival phases, so changes in the POMS mood factors may have been missed. The same applies to students' state of anxiety. Dramatic increases in anxiety during the sea and arid phases and even on alternate days during the jungle phase may have been missed. Shortened versions of both the POMS and the STAI are available and it may have been preferable to use these more frequently during the present study.

The periods spent in-barracks suggest that SIgA:Alb may be a marker of recovery from intense physical activity and food deprivation. It would have been useful (but not practical) to continue monitoring the health, diet, immune function, and mood state of the students for the month following the course. Future studies are needed to assess the value of SIgA:Alb in gauging recovery from stressful military exercises.

It is recommended that future survival school courses provide some nutritional advice, which emphasizes the need to eat well while in barracks and to avoid alcohol. In particular students should be advised to eat more carbohydrate-rich foods such as breakfast cereals, breads, pasta, rice, and starchy vegetables, than they would normally eat at home when their level of physical activity is less. Students should be informed about the need to remain well hydrated and avoid sunburn.

The results presented here show that SIgA:Alb is a useful marker of the severity of stresses encountered during the survival school course. This simple saliva test may be used as a tool for evaluating the effectiveness of interventions such as improved diet and measures designed to reduce fatigue. Further research is needed before SIgA:Alb can be recommended as a marker in the evaluation of positive psychological interventions.

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