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ABSTRACT

SIgA is a potential biomarker for stress. The usual day-to-day and within day variation in sIgA amongst a group of healthy Army reservists was estimated and the acute response of sIgA to moderate intensity exercise (Combat Fitness Assessment) undertaken in both cool-dry and hot-humid conditions was determined. The results indicate that thermal and cardiovascular strain resulting from moderate intensity exercise in hot-humid conditions suppresses sIgA for at least 24 hours post-exercise. Salivary sIgA exhibits a wide biological variation which casts some doubt on its usefulness as a biomarker, however because sIgA has been shown to be sensitive to dietary restriction, alcohol consumption, loss of body mass, fatigue and negative emotions in previous studies and now heat-induced cardiovascular strain, further work is warranted to develop this biomarker.

RELEASE LIMITATION

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Salivary Immunoglobulin A (sIgA) as a Biomarker of Immune Suppression Following the Combat Fitness Assessment

Executive Summary

Australian Defence Force (ADF) personnel are at risk of heat stress and symptoms of overtraining which can increase susceptibility to infection. The ADF Health Status report 2000 targeted the health of deployed forces as a priority for implementation of a health prevention program. The major problems experienced by this group include skin infections, injuries, upper respiratory tract infection (URTI), gut infection and unexplained disease or fever. Furthermore, the report states that the high usage of medications for treating coughs, colds and infections indicates that URTI are a significant source of illness within the ADF.

Secretory IgA is found in saliva and it is an indicator of increased disease risk. The study reported here is the second of a series designed to determine the usefulness of salivary sIgA as a biomarker of stress and an aid to improving the effectiveness of training programs.

The first phase of the study determined the usual day-to-day and within day variation in sIgA amongst a group of healthy Army reservists over eight weeks. The second phase of the study reported the acute response of sIgA to moderately vigorous physical activity (Combat Fitness Assessment) undertaken in both cool/dry and hot/humid conditions. The results indicate that thermal and cardiovascular strain resulting from moderately vigorous physical activity in hot-humid conditions suppresses sIgA for at least 24 hours post-exercise. Salivary sIgA exhibits a wide biological variation which casts some doubt on its usefulness as a biomarker, however because sIgA has been shown to be sensitive to dietary restriction, alcohol consumption, loss of body mass, fatigue and negative emotions in previous studies and now heat-induced cardiovascular strain, further work is warranted to develop this biomarker.

The long-term effect of post-exercise mucosal immune suppression such as that reported here, is not known. Because high incidences of URTI have been reported amongst deployed military personnel it is reasonable to propose that failure to recover from post-exercise mucosal immune suppression as a result of inappropriate work-rest cycles, might be a causal factor.

Further work is required to ascertain whether ADF work-rest cycles for typical military activities, particularly those conducted under the hot conditions of northern and central Australia may have a deleterious effect on immune functions.

Authors

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Paul graduated with a Bachelor of Human Movement degree with First Class Honours (majoring in Exercise and Sport Science) from the University of Tasmania at the end of 2001. He was also a recipient of the University Medal, the highest academic achievement award of the University, and an APA scholarship. He will shortly embark on post-graduate (PhD) studies.

Christine Booth
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Christine graduated from the University of Queensland (UQ) with BSc(Hons) and PhD (1992) in biochemistry (enzymology - cofactors and vitamins). She has also obtained qualifications in education (Dip Ed, UQ) and dietetics (Grad Dip Nutr Diet, QUT). She has membership of the Australasian Association of Clinical Biochemists, American Association of Clinical Chemists, Dietitians' Association of Australia, Nutrition Society of Australia (Secretary Tas branch), the Australian Institute of Food Science and Technology, Tasmania's Food Advisory Council and is an Honorary Research Associate in the School of Human Life Sciences, University of Tasmania. Christine has held research positions within UQ and QUT and a supervising scientist position within Chemical Pathology at Royal Brisbane Hospital. In her four-years employment as senior chemist at the Defence Nutrition Research Centre Christine has been investigating the nutritional status of soldiers and the effects of long-term combat rationing on health and military performance.

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Dan graduated with an honours degree in exercise science from Griffith University, Queensland. He commenced PhD studies at the same university in 1998, where his work focussed on fatigue in the central nervous system during exercise and after training. Presently Dan is a lecturer in exercise science at the University of Tasmania where he also supervises postgraduate students.

Contents

1. Introduction

1.1 Salivary Immunoglobulin A as a Biomarker

Decreased concentration of secretory immunoglobulin A (IgA) is a potential indicator of increased disease risk. Secretory IgA is found at the mucosal membranes, and represents one of the defences against viruses and bacteria. As most infectious agents enter the host via the mucosal membranes, a secretory IgA deficiency represents a reduced level of protection for the body, and an increased risk of infection.

Saliva, tears, nasal associated fluids, bronchial, intestinal and genitourinary secretions contain immunoglobulins, predominantly secretory IgA [1]. Secretory IgA protects the mucosal surfaces by several mechanisms, resulting in the inability of pathogens to attach to mucosal surfaces, thereby preventing entry into the body, limiting viral replication or aiding in elimination [2]. Secretory IgA concentrations are correlated with resistance to certain viruses responsible for upper respiratory tract infections (URTI) [3].

Due to the ease of saliva collection, salivary secretory IgA (sIgA) is the most convenient source for measurement of secretory IgA in field settings. Using sIgA as a biomarker of stress may be feasible if a robust and cheap method of analysis can be developed. Because, antisera to human IgA are readily available and sIgA has been shown to be stable under the conditions of northern Australia (unpublished in-house results), the development of a field method should be possible.

1.2 Exercise, Salivary Immunoglobulin and Increased Risk of Infection

Regular moderately vigorous physical activity has been shown to be of benefit of health [4]. The benefits of regular exercise are the result of a complex interaction between physiological and psychological factors [5]. Presently much research in this area is focused on the interaction between exercise and immune function. There is much anecdotal and epidemiological evidence that regular moderately vigorous physical activity has positive effects on immune function, for example by enhancing resistance to infections such as the common cold [6]. However, very intense physical activity is associated with both transient and chronic suppression of several immune factors including sIgA. In 1982 Tomasi and co-workers were the first to observe decreased concentrations of sIgA in saliva apparently as a result of cross country skiing [7]. Many similar observations have been reported since then for various sports [e.g. 7, 8, 9, 10, 11]. The evidence to date suggests that high-intensity physical activity is associated with a transient suppression of sIgA and that over many years regular intense physical activity training is associated with a chronic suppression of sIgA [12]. Certainly, athletes undergoing high intensity training have a high incidence of URTI [13, 14].

In Australia physical activity is often performed in adverse environmental conditions such as excessive heat and humidity, with consequent fatigue, exhaustion and heat stress [15].

Exercising, particularly in the heat, may cause hyperthermia (a rise in body temperature), which has been reported to have immunosuppressive effects [16], many of which are similar to the changes observed in relation to physical activity [17]. Unfortunately, little information is available regarding the effects of heat and humidity on sIgA concentrations, even less so when combined with physical activity.

Athletes face a variety of stressful situations. Exposure to psychosocial stressors [18], negative mood [19], daily hassles [20], anxiety [21] and exam stress [22] have all been shown to be associated with immune suppression. As with intense physical activity, psychological stress has an apparent suppressant effect on sIgA with consequent increased susceptibility to infections [21].

Few studies have investigated the effect of prolonged physical exertion on immune function in the military context. Military personnel may be exposed to high physiological and psychological stresses, during both training and operations. If this is combined with the heat stress and load carriage, it is reasonable to expect that service personnel could be subject to immune suppression.

Two trials which assessed US soldiers undergoing the rigorous special forces selection course (21 days) and the 64-day Ranger selection course revealed suppressed immune function, which was partially ameliorated by increased energy consumption in the latter study [23, 24]. Airfield Defence Guards undertaking routine training, which involved sustained moderately vigorous physical exertion, in Far North Queensland, experienced up to 50% decreases in sIgA to albumin ratio (sIgA:Alb) [25] as did participants of a Royal Australian Air Force Survival School course [26]. Energy restriction, and in particular inadequate carbohydrate consumption, were shown to be associated with the decreased sIgA in these two studies. However, in both situations the participants were subjected to extremes of environment, sustained physical work, fatigue and psychological stress as well as energy restriction and it is not possible to distinguish the separate effects of these variables. In the case of the survival school participants, low sIgA:Alb on the first day of the course was shown to be associated with an increased incidence of URTI during the year preceding the course and a low mean sIgA:Alb during the course was correlated with an increased incidence of URTI.

The evidence presented above leads to the conclusion that arduous physical work, adverse environmental conditions, psychological stress and energy restriction all have the potential to be immunosuppressive and that sIgA shows potential as a convenient biomarker of immune suppression.

1.3 Prevention and Detection of Excessive Stress

Excessive stress (physical and mental) can results in decreased military performance, but other symptoms can include fatigue, mood disturbances—including apathy, irritability, anger and depression—frequent URTI, injury, muscle soreness and joint pain. Many of these symptoms are evident during ADF field exercises [25, 26] and have been associated

with immune suppression. Upon enlistment, many ADF recruits may have less than average physical fitness. Indeed, a group of soldiers from 3 Brigade, Townsville were found to have an average aerobic fitness only marginally above that of their peers in the general Australian population [27]. Thus it is not surprising that physical training in a hot Australian climate, coupled with negative psychological factors and food restriction, might be stressful, particularly for ADF recruits, and may result in the symptoms described above, which are often referred to as 'overtraining'. For example, a study of US army recruits performing an 8-week physical training program found that about one-third could be classified as overtrained [28]. In physically fit special forces personnel, after 10 days of twice-daily maximal running, all subjects reported symptoms of overtraining such as inability to maintain training load, nausea, muscle soreness and irritability [29].

Physical activity exacerbates the rise in body temperature in any given environment. For example, a combination of heat stress and blistered feet resulted in a high failure rate (71%) in a combat fitness assessment conducted in northern Australia [27]. Military personnel in situations where protective full body cover is worn also face the problem of heat stress [15]. Depending on the material, the extra sweat tends not to evaporate through the clothing, because of low vapour permeability of the material, leading to discomfort and heat stress [30]. According to Shephard and co-workers heavy training schedules, the carrying of 30+ kg packs and the use of protective equipment, including clothing, have continued to exact a toll of heat illness among military personnel in recent years [31].

It appears that ADF personnel are at risk of heat stress and symptoms of overtraining and as discussed above, this can lead to immunosuppression and increased susceptibility to infection. The *Australian Defence Force Health Status Report* targeted deployed forces health as a priority for implementation of a health promotion program [32]. The major problems experienced by this group include skin infections, injuries, URTI, gut infection and unexplained disease or fever. Furthermore, the report states that the high usage of medications for treating coughs, colds and infections indicates that URTI are a significant source of illness within the ADF.

Although it is impossible to counter the effects of all the causes of training-induced immunosuppression, it is possible to minimise the effects of many of the contributory factors and thereby reduce the risk of minor infections and symptoms of overtraining [15, 33, 34, 35, 36, 37, 38]. Pyne [36] provides a useful summary of recommended approaches to the prevention of overtraining symptoms.

In support of ADF health promotion, Milestone 7 of Task ARM 01/067 aims to determine the usefulness of sIgA as a biomarker of unacceptable stress and an aid to improving the effectiveness of training programs. Ultimately the aim of human factors work within Combatant Protection and Nutrition Branch is to develop physiologic strategies to protect and sustain deployed soldiers, thereby enhancing operational readiness.

1.4 Purpose of the Study

The clinical chemistry aspects of the sIgA assay have not been well documented in the literature and it is likely that much of the conflicting evidence concerning the effects of mood and physical activity on sIgA is a result of non-standardised assay procedures. There is some controversy concerning the best way to express sIgA concentration. Salivary secretory IgA concentration can be expressed as sIgA concentration (μg/mL), sIgA to salivary albumin ratio (sIgA:Alb) and sIgA secretory rate (μg /min). The first aim of the study was to estimate the biological variability of the three sIgA measures using our current laboratory assay procedure. Based on these calculated values, the feasibility of using an estimated healthy reference interval for the measures was to be determined.

The second aim of the present study was to examine the acute sIgA response of healthy adults to a typical military activity requiring moderately vigorous physical activity. The exercise protocol was modelled on the ADF's combat fitness assessment (CFA), which requires soldiers to march a set distance at a prescribed pace in patrol order (total load 20 kg). Because exercising in a hot and humid climate provides additional physiological stress that may contribute to changes in mucosal immunity [16], this study also aimed to evaluate the sIgA response as a result of the aforementioned activity in two environmental conditions (hot/humid versus cool/dry), similar to those soldiers might experience in operational situations.

1.4.1 Assumptions

It was assumed that the test subjects were representative of the Army reservist population, that they were compliant with all the saliva sampling procedures and that their sIgA response was indicative of the expected response from the wider Army population. It was also assumed that all subjects completed the various written assessment instruments accurately and that these tests were valid.

1.4.2 Limitations and delimitations

1. A small cohort of Tasmanian Army reservists and university students (15 males and 6 females) were used as test subjects. They were screened for factors such as age, medical history, nutritional status and fitness level. The narrow definition of the cohort means that they might not be representative of the general Army population. Nevertheless, the external validity of this experimental design is enhanced because subjects were randomly selected.
2. All subjects lived in Tasmania and were not accustomed to physical activity in a hot/humid climate, therefore their response to the test conducted in hot conditions is applicable only to Army personnel who deploy from a cold climate to a hot/humid operational area and engage in physically arduous operations before becoming heat-acclimatised.
3. Although a review of the literature indicated equivocal evidence for diurnal variation in sIgA, it is prudent to conduct all testing at the same time of day. This

was not possible because of time constraints imposed by the subjects. This limitation was partially mitigated by conducting the testing at the same time of day for each subject. A pilot study using four male and two female subjects was conducted to examine possible diurnal variation in sIgA.

4. The validity of the various written and verbal assessment instruments relied on the compliance of the test subjects and the accuracy of their answers. Test subjects were thoroughly briefed on their proper use. Some of the instruments (e.g. RPE estimate of effort) were backed up by heart rate measurements.

5. Although rectal body core temperature measurement is accurate, it is also invasive and unpopular with test subjects. Therefore tympanic (ear) temperature was measured. Although good correlations between these two estimations of body core temperature have been reported [39, 40, 41], in this study tympanic temperature was used only as an indicator of possible heat stress. The results are reported for the sake of completeness, but they are not an integral aspect of this study.

6. Because of time constraints the subjects' fitness levels, as indicated by their aerobic capacity ($\dot{V}O_{2max}$), were not measured by the usual gas-exchange method, which is accepted as the gold standard. Rather $\dot{V}O_{2max}$ was estimated by use of the multistage fitness test. This method has been shown to have an acceptable correlation with laboratory-based $\dot{V}O_{2max}$ tests (r = 0.90) [42].

7. The accuracy and reliability of sIgA measurements depends on the compliance of test subjects with regard to proper collection of the sample, labelling of the sample tube, and storage and transport of the sample as well as the precision and accuracy of the assay method. To this end subjects were provided with both verbal and written instructions and the assay procedure was carried out by an experienced operator within a laboratory which is ISO9001 certified. Furthermore, each subject's samples were batched and then analysed within the same analytical run.

8. The temperature and humidity of the cool/dry environmental conditions could not be controlled. However, this phase of testing was conducted during winter and the ambient temperature was suitable, averaging 19 °C fluctuating only within a 3 °C range.

2. Methods

2.1 Subjects

Eleven females and 27 males from Paterson Barracks (16 Field Battery and 10th Field Ambulance) and Youngtown Barracks (12/40 RTR, A company) in Launceston Tasmania initially volunteered for this study. Each subject's suitability for the study was determined by screening for medical and dietary history and physical fitness. Medical screening was by use of the Physical Activity Readiness Questionnaire (PAR-Q, Appendix 1), which has been recommended as a minimal standard for entry into moderate-intensity exercise programs by the American College of Sports Medicine [5]. Dietary screening was by use of a food frequency form (Section 2.2) and the physical fitness test was the walking or

running component of the Army's basic fitness assessment (BFA). Contraindications for inclusion in the study were:

1. An inability to complete the walking (5 km) or running (2.4 km) component of the BFA in the required time based on age and gender.
2. Adverse medical history as reported in the PAR-Q.
3. Extreme dietary habits such as evidence of fad diets.
4. Pregnancy or the intention to become pregnant during the study period.
5. A body mass index (BMI) below 18.5 (underweight) or above 30 (obese).
6. Hypertension: a resting blood pressure above 140 mm Hg (systolic) or 90 mm Hg (Diastolic) [5].
7. A history of regular physical activity of greater than two hours per day or intensive training for a competitive endurance sport.

Subjects who were accepted into the study received written notification and were requested to avoid making changes to their diet or exercise routine for the duration of the study. Each of the original volunteers was given the results of their dietary analysis with some relevant nutritional advice. Seven of the original volunteers were rejected in accordance with the guidelines above and 10 others withdrew from the study for personal reasons. Six females and 15 males completed the study. The mean age of the study cohort was 26.9 years with a range of 18.1 to 54.5 years.

Pilot study: Six volunteers (2 female, 4 male, age range 23–51) were drawn from the scientific team and staff members of the Defence Nutrition Research Centre (DNRC). These volunteers were not subjected to the selection criteria outlined above.

The experimental procedures were approved by the Australian Defence Human Research Ethics Committee (ADHREC protocol 182/99). Written consent was obtained from each participant after the details of the study were explained. Copies of the information and consent forms are included in Appendix 2.

2.2 Dietary intake measurement

A food frequency questionnaire, which had previously been validated against a three-day food diary [44], was used to examine dietary habits. Nutrient intake was calculated using the Foodworks calculation software (Version 2.05, Xyris Software, QLD) with the AusNut, AusFoods and Nuttab95 food composition databases.

2.3 Physiological measurements

2.3.1 Multistage fitness test

This test, commonly referred to as the "shuttle run" or "beep test", was conducted to estimate subjects' aerobic capacity [45]. Subjects were required to run back and forth between two lines 20 m apart at progressively faster speed until volitional exhaustion.

Subjects were guided through this protocol by matching their shuttle runs with audible beeps. Each subject's final speed was used to estimate aerobic capacity by use of a regression equation [45].

2.3.2 Heart function

During the treadmill stress tests heart function was monitored continuously by three-lead electrocardiogram (ECG, Kenz-Cardioscope 2016, Suzuken Co. Ltd., Nagoya, Japan). This non-invasive painless device uses three adhesive electrodes on the chest to measure the electrical activity of the heart. Electrodes were placed in the following anatomical locations: electrode 1 on the right clavicle, electrode 2 on the lower right rib along the mid-clavicular line and electrode 3 in the left 5th intercostal space on the anterior axillary line [5].

2.3.3 Body core temperature

As a safety measure, during the treadmill stress tests, body core temperature was estimated by tympanic temperature measurement, using a commercially available device (Thermoscan IRT 3520, Type 6013, Braun, Kronberg, Germany). This thermometer measures the temperature of the typanum (eardrum) by using infrared radiation. This method is less invasive, but also less accurate than rectal or oesophageal temperature measurements. The technique has been reported to be more accurate than axillary or oral temperature measurements when purpose built, research quality tympanic thermometers have been used [46], and the method has been has validated against oesophageal and rectal temperature measurements in various conditions, including under environmental heat stress [39, 40, 41, 47]. However, the accuracy of off-the-shelf tympanic thermometers is questionable (Mark Patterson, CPNB-Maribyrnong, pers. comm.). The results reported below are not considered to indicate the actual body core temperatures; rather they are an indication of the relative levels of heat strain experienced by subjects in the different environmental conditions.

2.3.4 Body composition

Body mass (kg) was measured by use of calibrated scales (AND, model CH-150K, A&D Mercury Pty. Ltd., Australia). Subjects were dried then weighed in underwear after voiding their bladders.

Bioelectric impedance was measured by use of the Seac BIM 4 bioelectric impedance analyser (BIA) (Seac Pty Ltd and Uniquest Ltd, QLD, Australia). The instrument uses a single electric frequency of 50 kHz and uses the Lukaski prediction equation to estimate percentage body fat from a calculated estimate of total body water [16]. A calibration check was performed immediately before each measurement session and if necessary the instrument was recalibrated [48].

The bioimpedance method is based on the 'two compartment' theory of body composition: that the body can be divided into 'fat mass' (FM) and 'fat free mass' (FFM). Water and electrolytes (which conduct electricity) are found only in the FFM. Hence the extent of impedance of an electric current will depend on the relative proportions of FM and FFM in the body. A very weak current (~200 μA, which is undetectable by the subject) is passed through the body and the resulting impedance is used to estimate FM and FFM.

Subjects were tested in a well-hydrated post-prandial state. Subjects were instructed to eat a carbohydrate-rich meal before testing and to drink fluids throughout the day, including a large glass of water at least an hour before measurement with no further drink or food within one hour of measurement. The subject lay face-up with legs slightly apart and hands resting next to the trunk, palms down. Care was taken to ensure that the hands were not touching any part of the body. The inner thighs were not permitted to be in 'skin-to-skin' contact. The subject had removed his/her right shoe and sock. Electrodes connected to the BIA were placed on the right hand and right foot as described in the manufacturer's instruction manual.

2.3.5 Urine specific gravity

Urine samples collected before and after the treadmill stress tests were tested for specific gravity (SG) by use of a disposable dip-stick (Multistix 10SG, Bayer Diagnostics Manufacturing Ltd., Bridgend, UK). Urine samples were collected from mid-stream flow into sterile containers.

2.4 Saliva tests

Subjects were asked to rinse their mouth, swallow until dry and then place a cotton swab in their mouth, leaving it in place without chewing for exactly one minute. The subject then placed the cotton swab into a Salivette tube (Starstedt, Germany). Albumin (Alb) and sIgA were measured by nephelometric assay (Dade Behring BNA II) using manufacturer-supplied reagents (antisera to Human IgA α chain and human albumin). The results were presented as the ratio of sIgA (mg/L) to Alb (mg/L), sIgA secretion rate (μg/min) and as sIgA concentration (mg/L).

Samples collected from each phase of the study were batched and then stored frozen at -80°C until analysis. Samples from the first phase were analysed over three non-consecutive days and the samples from the second phase were analysed within the same day. To reduce analytical error, each subject's samples were analysed within the same day. Reagents from the same production batch were used throughout testing.

2.5 General health and physiological status questionnaires

2.5.1 Physical condition report

During both phases of the study subjects were asked to complete a simple health checklist ('physical condition report') which included the following health problems: cough, cold, runny nose or sore throat (suspected URTI), skin complaints (rashes or sores), dehydration, insect bites, headaches, allergic reactions, gut problems (stomach upset, nausea or vomiting), exhaustion, muscle or joint problems (fracture, dislocation or muscle strain), sunburn, cuts or bruises and other physical symptoms (Appendix 3). Each symptom was scored as *present* or *absent* at each time point to give a physical condition score of between 0 and 12.

2.5.2 Rating of perceived exertion (RPE)

Borg's subjective RPE [5], which is a reliable indicator of exercise tolerance, was used during the treadmill stress tests (Appendix 4). Perceived exertion ratings correlate highly with exercise heart rates and work rates. The RPE scale was developed to allow the exerciser to subjectively rate his/her feelings during physical activity, taking into account personal fitness level, environmental conditions and general fatigue levels. Physical activity intensity is rated on a scale of 6 (with 7 being very, very light) to 20 (with 19 being very, very hard).

2.5.3 Rating of thermal discomfort (RTD)

The RTD, which was administered during the treadmill stress study, is a 5-point subjective scale (Appendix 4). Discomfort was rated on a scale of 1 (comfortable) to 5 (extremely uncomfortable).

2.5.4 Rating of thermal sensation (RTS)

The RTS administered during the treadmill stress test allowed subjects to communicate how they perceived the thermal environment on a scale of 1 (unbearably cold) to 12 (unbearably hot) (Appendix 4).

2.5.5 Menstrual cycle questionnaire

Female subjects were requested on one occasion to complete a questionnaire indicating the phase of their menstrual cycle (Appendix 5).

2.6 Determination of biological variation – study phase 1

2.6.1 Pilot study- diurnal variation

There is no strong evidence in the literature that salivary secretion of sIgA has a defined diurnal variation. In order to confirm this, a pilot study was conducted whereby each of six subjects (two females and four males) collected saliva samples at up to ten timed intervals throughout a 24-hour period.

2.6.2 Biological variation

The first phase of the study was designed to determine the biological variation of sIgA. All subjects in the study cohort provided a timed unstimulated whole saliva sample on three mornings (before breakfast) each week for two months. Immediately after collecting each sample subjects completed the physical condition report. Female subjects completed the menstrual cycle questionnaire at the beginning of the study period. Salivary secretory IgA results obtained on days when subjects indicated that they were unwell were not included in the data analysis.

2.7 Treadmill stress test – phase 2

The second phase of the study was designed to investigate the acute effects of moderate intensity physical activity on sIgA under two environmental conditions. After completing the multistage fitness test for determination of $\dot{V}O_{2max}$ subjects completed two treadmill stress tests. Subjects were used as their own controls by performing the stress test in both environmental conditions with the order of the conditions randomised and separated by a period of six to 24 days. To avoid the confounding effect of diurnal variation in sIgA, tests were conducted at the same time of day for each subject.

2.7.1 Environmental conditions

The stress tests were performed in two environmental conditions that were chosen to reflect a hot-humid (HH) climate and cool-dry (CD) climate. The HH tests were conducted in a climate-controlled environmental chamber where the temperature was $28.1 \pm 0.2°C$ and the humidity was $80.0 \pm 0.9\%$ (the average Summer conditions of Innisfail, North Queensland as sourced from the Bureau of Meteorology). The CD tests were conducted in the ambient conditions of the human performance laboratory, where the temperature maintained at $19.0 \pm 1.3°C$ and the humidity was $51.0 \pm 8.0\%$.

2.7.2 Stress test protocol

Subjects completed a physical condition report before commencing each stress test, provided a urine sample for SG analysis and had their height, body mass (in underwear and in patrol order uniform), body composition (by BIA) and blood pressure measured before and after completing each stress test. Saliva samples were collected immediately

before commencement of the stress test (baseline), immediately following the stress test (post-test, time 0) and at 15 min, 30 min, 1 h, 2 h, 8 h and 24 h intervals.

Subjects were dressed in patrol order and carried a total of 20 kg (which included disruptive pattern combat uniform, standard issue webbing, backpack, water bottles and a replica Steyr rifle weighing 4.5 kg) above their body mass. The test was a modified version of the Army's combat fitness assessment (CFA). Subjects walked for two hours at a pace of 5 km per hour on a motorised treadmill (either a Quinton Q65, series 90, Quinton Instruments Co, Bothell USA or a Reebok ACD 3, Reebok, USA).

Heart function was continuously monitored and body temperature recorded at 30 min intervals. The RPE, RTD and RTS were completed by subjects at baseline, 30 min and 120 min. Subjects drank water *ad libitum* during the stress tests and were encouraged to drink a minimum of 1 L of water per hour during the HH treatment. Hydration status before and after the tests was determined by changes in body mass, urine SG and BIA. Tests were terminated at the discretion of the investigator or if any of the following occurred: an abnormal cardiac response [5]; heart rate exceeded 90% of the subject's age-predicted maximum for 3 consecutive minutes (i.e. HR_{max} = 220 − age); tympanic temperature exceeded 39.5°C [31] or the subject indicated that he/she could not continue, felt dizzy, nauseous or became disorientated.

2.8 Data analysis

2.8.1 Biological variation

The precision of the assay was determined by measuring the sIgA and Alb concentrations in a pooled human saliva sample with each analytical run. From these data the assay coefficient of variation (CV) was determined and hence the biological within-subject variance, the between-subject variance and the index of individuality were determined [49, 50]. From the combined dataset a healthy reference interval was estimated as the central 95% interval bounded by the 2.5 and 97.5 percentiles of distribution [51]. This interval represents only a rough estimate, because a cross-sectional study is required for an accurate determination of the reference interval.

2.8.2 Statistical methods

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 Spearman's ranked bivariate correlation were used to assess associations between variables. Comparison of means was achieved by use of the either the Students t test or paired t test

and Levene's test was used for comparison of variance. In order to determine the statistical difference between treatment groups, Univariate analysis (general linear model) with LSD post-test was applied to the baseline measurements of the two stress tests. Repeated measures analysis of variance, which was based on covariance-adjusted post-stress test responses, was used to compare environmental treatments for tests with serial measurements.

3. Results

3.1 Pilot study – diurnal variation in salivary secretory IgA

The individual results are plotted versus time of day in Figure 1. A visual inspection of each subject's results suggests a peak early in the morning after waking, reasonably stable values during the day, and a second smaller peak late in the evening. However, the data show a wide scatter of results for time of day and a repeated measures analysis of variance revealed no significant effect of time (e.g. for sIgA:Alb data, $F = 4.276$, $p = 0.132$).
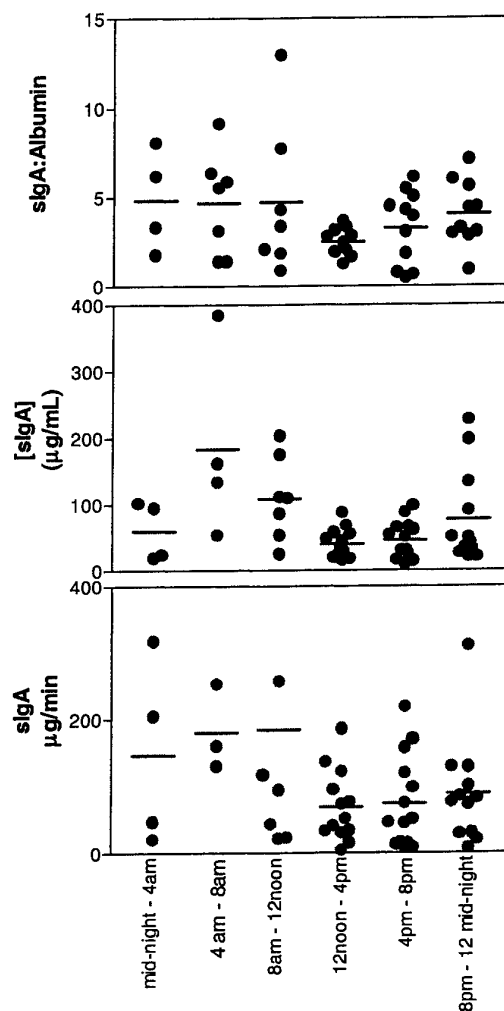

Figure 1 Individual measures of sIgA according to time of day. The mean for each time period is indicated

3.2 Biological variation – phase 1

The anthropometric data for the study cohort (21 subjects) is detailed in Table 1.

Table 1 Mean anthropometric data for the study cohort

Variable	Mean	SD	Range
Age (years)	26.9	9.5	18.1 – 54.5
Height (cm)	173.8	7.4	159.5 - 189
Body mass (kg)	72.4	10.9	54.3 – 105.8
Body mass index	23.9	3.2	20.3 – 34.9
% Body fat	21.2	7.7	8.1 – 32.8

Of the 438 returned saliva samples nine were discarded because of insufficient volume or contamination with food or blood. Samples collected from subjects who concurrently reported a symptom of URTI or other illness were not included in the analysis. Table 2 details the mean saliva test results for the cohort after outliers and results corresponding to self-reported URTI or other illness were removed.

Table 2 Mean saliva test results for the study cohort

	All samples		Males		Females	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
SIgA:Alb (mg/mg)	287	66.7 ± 32.6	194	68.8 ± 31.7	93	62.8 ± 34.0
SIgA (mg/L)	247	3.4 ± 1.9	163	3.4 ± 2.0	84	3.3 ± 1.4
SigA secretion rate (μg/min)	255	65.1 ± 31.1	166	75.6 ± 41.5	89	45.5 ± 33.5

There was no significant difference between male and female test results, although the difference in results for sIgA secretion rate approached significance ($t = 1.96, p = 0.07$). The sIgA measures did not change significantly with time over the two months (data for days of recorded URTI, removed). An examination of the dataset including days of illness revealed that Alb secretion rate did not change over time ($F=0.665, p = 0.725$). No correlations with sIgA measures were found between sIgA and either aerobic capacity or age. Spearman rank correlation indicated a weak negative association between measurement of sIgA and self-reported health problems (Table 3).

Table 3 Correlation between sIgA measures and ill-health

	Ill-Health[a]		URTI[b]	
	Correlation coefficient	Significance (2-tailed)	Correlation coefficient	Significance (2-tailed)
SIgA:Alb	-0.256	0.000	-0.12	0.015
SIgA secretion rate	-0.104	0.034	-0.119	0.015

[a] *Refer to the physical condition report section 2.5.1.*
[b] *URTI refers to upper respiratory tract infection.*

Because each subject's samples were analysed on the same day using the same batch of reagents, the intra-assay CV (4.51 for sIgA, 2.68 for sIgA:Alb) was used for subsequent calculations. Coefficient of variation could not be calculated for sIgA secretion rate because the quality control measurements used a sample of pooled saliva samples that were not from timed collections. The mean biological within-subject variance was 42.5% for sIgA concentration, 37.5% for sIgA:Alb and 44.9% for sIgA secretion rate and the between-subject variance was 48.8% for sIgA concentration, 54.9% for sIgA:Alb and 63.6% for sIgA secretion rate. The indices of individuality were 0.87 for sIgA concentration, 0.68 for sIgA:Alb and 0.59 for sIgA secretion rate. The estimated healthy reference ranges were: sIgA concentration (21.5–161.9 mg/L), sIgA:Alb (0.52–7.85) and sIgA secretion rate (6.24–162.9 µg/min).

3.3 Treadmill stress tests – phase 2

3.3.1 Physiological measurements

The mean aerobic capacity for the group was 43.1 ± 6.8 mL/kg/min with a range of 34.0 to 57.4. All subjects were found to maintain euhydration throughout the stress tests. The change in the hydration status measures of body mass and urine SG are detailed in Table 4. Subjects drank more water during the HH stress test and this was reflected in subjects having an increase in body mass and total body water, which was significantly greater than that for the CD stress test. Urine SG remained virtually unchanged for subjects under both environmental conditions. Mean post-test urine SGs were 1.010 ± 0.008 (range 1.005 to 1.030) for CD and 1.013 ± 0.009 (range 1.005 to 1.030) for HH. In each of the datasets (HH and CD) two subjects had a urine SG of 1.030, which indicates mild hypohydration. However hypohydration was not confirmed by body mass loss.

Body water estimates obtained by BIA measurement displayed a positive bias when compared with body mass measurements:

Body mass loss/gain (kg) = 0.45 x body water loss/gain (kg) – 0.4;
(linear regression: $r = 0.75$, $p < 0.001$)

For example, the estimated change in body water using this method was for the HH group 1.0 ± 0.9 kg and for the CD group, 0.6 ± 0.7 kg. Although the method may be suitable for

detecting changes in body water, these results highlight the inaccuracy of BIA measurements and prediction equations in determination of body water.

Table 4 Mean changes in measures of hydration status according to environmental condition

	Hot/humid Mean ± SD	Cool/dry Mean ± SD	Paired t test results	
			t	p
Body mass loss/gain (kg)	0.61 ± 0.9	-0.06 ± 0.55	3.320	0.004
Urine SG (g/mL)	-0.004 ± 0.001	-0.005 ± 0.001	ns	ns
Water consumption (L)	2.56 ± 1.10	1.54 ± 0.66	5.648	0.000

No significant levels of cardio-vascular or thermal strain were experienced during the CD stress test and only moderate strain was experienced during the HH stress test.

Mean baseline tympanic temperatures were not different for the two stress tests (36.7 ± 0.6°C for HH and 36.5 ± 0.4°C for CD). Rise in tympanic temperature during the HH stress test (38.0 ± 0.8°C, range 36.5–39.5 °C, t = 10.531, p < 0.001) was greater than the CD stress test (36.6 ± 0.5°C , range 35.3 – 37.4 °C, non-significant).

From the ECG graphs it was evident that for most subjects heart rate remained more or less steady-state during the CD march but climbed steadily during the HH march, rising from 111 ± 13.6 bpm after 1 minute to a peak of 159 ± 14.0 bpm at the finish. Table 5 presents heart rates expressed as a percentage of subjects' age-predicted maximum heart rate. Heart rates were significantly higher for HH throughout the two-hour march except at the start (t = 11.789, p = 0.000 at 2 h).

Table 5 Heart rate relative to calculated age-predicted maximum[a]

Time	HH		CD	
(min)	Mean & SD	Range	Mean & SD	Range
0	58% ± 7%	44% - 69%	55% ± 6%	43% - 66%
60	74% ± 7%	66% - 96%	62% ± 6%	52 – 79%
90	78% ± 9%	67% - 105%	64% ± 7%	49% - 76%
120	83% ± 9%	69% - 109%	67% ± 8%	56% - 86%

[a] *Age-related maximum heart rate calculated as 220 – age (beats per minute).*

3.3.2 Psychophysiological status

Results of the RPE, RTD and RTS are presented in Table 6. All ratings increased throughout both the HH and CD treatments. Ratings of thermal sensation were always significantly higher for HH (p = 0.000). Ratings of thermal discomfort and RPE were significantly higher for HH at the halfway point and at the end of the test (p < 0.001).

Table 6 Mean responses to physiological status questionnaires during the stress tests

	Time (min)	HH	Mean ± SD	CD	Mean ± SD
Rating of Thermal Sensation	0	Mildly warm	7.90 ± 0.45	Slightly cold to neutral	6.55 ± 1.00
	60	Hot	9.10 ± 0.90	Slightly warm	8.08 ± 0.89
	120	Hot to very hot	9.65 ± 1.09	Slightly warm to hot	8.48 ± 0.82
Rating of Thermal Discomfort	0	Slightly uncomfortable	1.65 ± 0.59	comfortable	1.35 ± 0.59
	60	uncomfortable	2.98 ± 0.87	Slightly uncomfortable	2.00 ± 0.73
	120	Very uncomfortable	3.50 ± 0.89	uncomfortable	2.38 ± 0.96
Rating of Perceived Exertion	0	Fairly light	10.80± 1.61	light	10.30± 1.17
	60	Somewhat hard	13.63± 1.51	Fairly light	12.20± 1.44
	120	Hard	14.90± 2.38	Somewhat hard	13.15± 2.13

The psychophysical responses appear to be greater than the observed thermal and cardio-vascular strain and may be indicative of poor acceptance of high skin wettedness. This poor perceived thermal tolerance is not unexpected in this population considering the usual Tasmanian winter climate. However, the results also reflect the limitations of the tympanic temperature measurement, which underestimates true core body temperature. Safety of subjects was not compromised by the failure of the tympanic temperature measurement, because the other safety considerations—heart rate, visual inspection and voluntary termination by the subject, still applied.

3.3.3 Salivary secretory IgA measures

Univariant analysis of baseline data showed that there were no differences in any of the sIgA measures (ie concentration, secretion rate or ratio) at the start of the two tests. For each stress test subjects were required to collect eight saliva samples, four of which were to be collected at home. Because many of the latter samples were not returned to the laboratory and several results were removed as outliers, the complete dataset for the measures of sIgA was reduced to n = 5 to 9 for each test. In contrast the dataset for the first four time points (samples collected in the laboratory) was nearly complete and was therefore also used for the statistical analyses.

The effects of gender and environment were not significant. However, in the case of the sIgA:Alb measure the immune suppression evident during the HH stress test almost reached significance compared with the CD stress test results in the first 60 min (Between-subjects effect, $F = 4.137$, $p = 0.057$, Figure 2).

Salivary IgA content changed significantly with time after the stress tests (Tables 7 and 8, Fig 2). For sIgA concentration and sIgA secretion rate the values were significantly elevated above baseline at the 15 min and 30 min time points then returned to baseline values. A suppressed sIgA:Alb response was recorded at zero time after both CD and HH stress tests, and in the case of the CD treatment values returned to baseline level by 2 hours, but remained suppressed for 24 hrs after the HH treatment.

The salivary measures, concentration, secretion rate and ratio, responded differently over time ($F = 19.6$, $p = 0.000$, data for the two stress tests averaged, $n = 18$) with albumin concentration and sIgA:Alb being different to all other measures (LSD post-hoc analysis, all $p < 0.05$) and sIgA concentration and secretory rate showing a similar response.

Table 7 Effect of time for stress tests conducted in hot/humid and cool/dry environments

	8 time points			First 4 time pts		
	F	p	n	F	p	n
Albumin	2.165	0.237	12	13.805	0.000	32
sIgA	2.768	0.024	20	10.7	< 0.01	34
sIgA:Alb	0.801	0.539	21	2.309	0.082	35
sIgA secretion rate	4.149	< 0.01	18	8.070	< 0.01	32
Albumin secretion rate	1.769	0.194	18[a]			
Saliva flow rate	2.552	0.10	17	5.456	0.004	34

[a]*Repeated measures analysis of albumin secretion data omitted the time point immediately before commencement of the stress tests, because these data were non-normal even after natural log transformation.*

Table 8 Results(mean ± SEM) averaged over the two stress tests

	Timed collection points							
	Pre-test	0 min post-test	15 min	30 min	60 min	2 h	8 h	24 h
Albumin (mg/L)	23.5 ± 3.2	28.3 ± 4.4	38.0 ± 6.0	43.2 ± 5.9	36.4 ± 7.6	36.0 ± 7.6	35.1 ± 7.5	28.9 ± 4.7
SIgA (mg/L)	56.0 ± 3.9	55.9 ± 6.0	77.5 ± 9.2	87.4 ± 10	52.5 ± 6.2	60.4 ± 8.3	70.1 ± 9.8	62.9 ± 9.5
sIgA:Alb (mg/mg)	3.06 ± 0.3	2.56 ± 0.24	2.69 ± 0.3	2.74 ± 0.3	2.03 ± 0.3	2.97 ± 0.5	2.7 ± 0.3	2.78 ± 0.3
sIgA secretion rate (μg/min)	59.0 ± 7.4	43.5 ± 5.6	59.5 ± 7.1	70.0 ± 7.5	55.2 ± 7.4	63.3 ± 7.5	66.4 ±10.6	61.4 ± 9.1
Albumin secretion rate (mg/min)	21.8 ± 2.9	22.5 ± 4.3	29.2 ± 4.7	32.3 ± 4.2	40.5 ± 10	42.8 ± 10	34.1 ± 9.8	31.4 ± 6.7
Saliva flow rate (mL/min)	0.98 ± 0.07	0.77 ± 0.06	0.79 ± 0.07	0.80 ± 0.06	1.11 ± 0.09	1.23 ± 0.09	0.92 ± 0.08	1.05 ± 0.10

Linear regression analysis (Table 9) shows that sIgA secretion rate is the sIgA measure most sensitive to salivary flow rate and that sIgA:Alb was least sensitive to flow rate. Albumin, sIgA and sIgA secretion rate were also sensitive to a measure of hydration status, % body water ($r = -0.323$, -0.340 and -0.380 respectively, all $p < 0.05$) whereas sIgA:Alb was not correlated with % body water.

Table 9 Linear regression with flow rate as the dependent variable

	Slope[a]	r	F	p	n
Albumin	-1.1 ± 3.4	0.019	0.11	0.74	294
sIgA	-25.4 ± 4.8	0.296	28.3	< 0.0001	297
SIgA:Alb	-0.7 ± 0.2	0.234	17.2	< 0.0001	298
SIgA secretion rate	-54.0 ± 8.1	0.359	43.8	< 0.0001	298

[a] *The 95% confidence interval is indicated.*
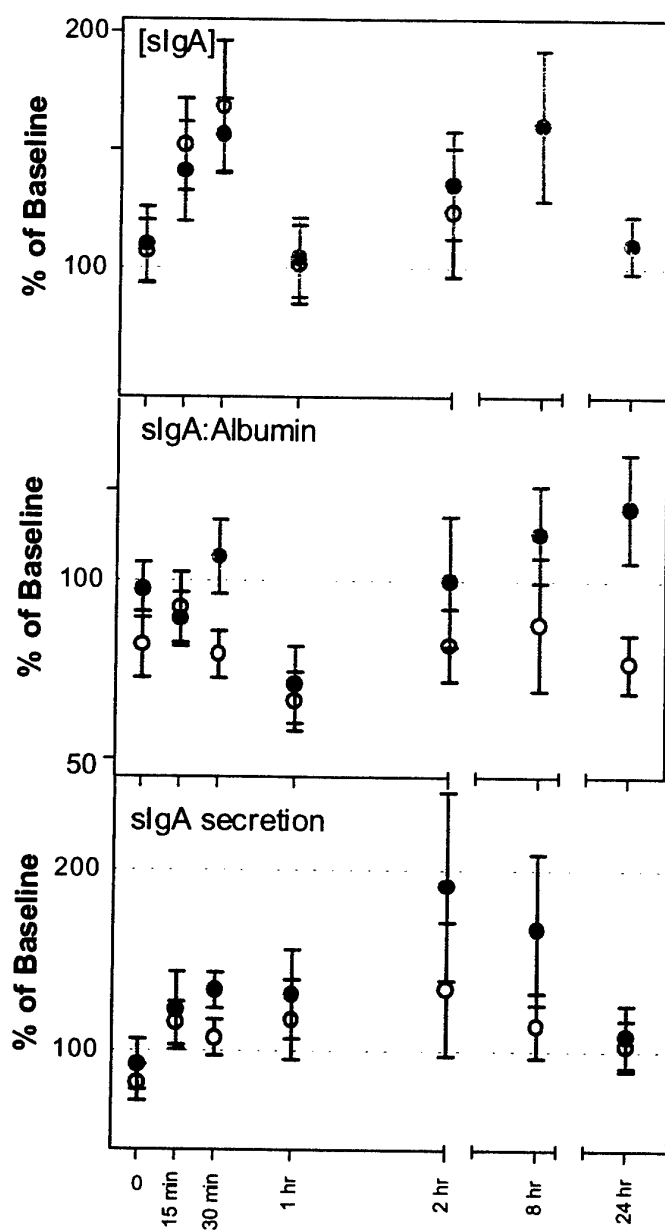
Figure 2 Effect of environmental treatment on measures of sIgA. Hot/humid treatment (o), cool/dry treatment (•), and SEM (⊥) are indicated

4.1 Assay performance and biological variation

The criterion for acceptable assay precision is that the assay CV should be less than half the within-subject variation (Results section 3.2, CV = 2.68, 4.51 for sigA:Alb and sIgA

respectively compared with biological variation = 37.5, 44.9 respectively) [49]. Although the intra-assay precision for the immunoassay appears acceptable the inter-assay precision in our laboratory, particularly for differing batches of reagents, has historically been poor with CVs up to 12% (results not reported, here). This is mostly due to the age of our present instrument which has been recording high background and hence reduced sensitivity in the lower range for the assay. Error in the collection of samples, particularly in the case of the timed collection and the reduced sensitivity of our present method may explain some of the calculated biological variation, but regardless of these problems the biological variation is still large. The higher biological variation for sIgA concentration compared with sigA:Alb may reflect the greater sensitivity of sIgA concentration to salivary flow rate.

Direct comparison of published effects of physical activity on mucosal immunity is made difficult by the lack of uniformity in measuring and expressing sIgA. For example there are differences in assay method, calibration material and specificity of the antisera. In 1994 a certified reference material (CRM 490) was prepared by the International Federation of Clinical Chemists (IFCC). The calibration standard used in our assay is referenced against CRM 490. A literature search revealed that not all recently published reports have referenced CRM 490. Hence reported values for sIgA vary greatly, for example 170–200 µg/min (average) and 78 ± 67 µg/min (SD = 86%) in studies reported by two laboratories [22, 9]. Published results from a German laboratory using reagents and instrumentation similar to our laboratory reported sIgA:Alb for 15 athletes immediately after completion of a marathon race as 3.3 ± 4.3 (range 0.3–14.0) which was consistent with the range of results recorded immediately following the treadmill stress tests (2.56 ± 1.2, range 0.26–5.90). It is probable that technical error and non-standardisation of assay methods contributes greatly to the large SDs and inter-laboratory differences in reported sIgA ranges.

Not only was a high biological variation recorded for our study cohort, but also a high degree of individuality (Results section 3.2: 0.87, 0.68, 0.59 for sIgA concentration, sIgA:Alb and sIgA secretion rate respectively). If the index is < 0.6 then a high degree of individuality exists for the measurement within the examined population. This means that the determined reference interval will not be very helpful for detecting significant change in serial measurements for an individual. Reference intervals are most helpful when the index is > 1.4. However, in biology this is rarely the case. This means that subjects may have an unusual measurement result for them, but still fall within the healthy reference interval [49]. For example, with the present cohort a change of 3.17 would represent with 80% confidence a significant change in sIgA:Alb for a subject, based on the calculated healthy reference interval. Only one subject experienced a decrease in sIgA:Alb of this magnitude in response to the HH stress test. Whereas, based on individual SDs calculated over the two month recording period, eight individuals had significant decreases in sIgA:Alb in response to the HH stress test.

Salivary secretory IgA can be expressed as concentration, secretion rate and as a ratio to total protein concentration, albumin concentration or osmolality. Most published reports have used sIgA concentration (µg/mL of saliva). Because both saliva flow rate, which is

commonly reduced by exercise-induced dehydration, and evaporative loss of saliva water, which occurs with increased oral breathing during physical activity, can have a concentrating effect on saliva solutes, sIgA concentration results can be misleading [43, 52]. Hence decreased sIgA output and transport during physical activity may not be detected.

Expressing sIgA concentration against a salivary marker which isn't actively transported and therefore is insensitive to most physiological changes, should account for changes in saliva water volume [53]. Total salivary protein and salivary albumin have been used for this purpose. The use of total protein has been criticised because certain proteins, which make up a significant proportion of salivary protein content, are sensitive to physiological conditions (e.g. salivary amylase and other immunoglobulins) and may influence total protein secretion rate [2]. Of these two markers albumin would be preferable because it is passively transported into saliva from plasma, where it is under homeostatic control (35-50 g/L or ~0.6 mmol/L). However, changes in the intravascular plasma albumin concentration as a result of postural change, pregnancy, congestive heart failure, severe malnutrition or liver failure affect plasma concentration. Postural change from seated to standing can increase plasma albumin concentration by 10–15% as water is lost from vessels of the lower extremities due to increased hydrostatic pressure [54]. The effect these conditions may have on salivary albumin concentration is not known.

Another potential marker of saliva water volume change is osmolality. Because inorganic electrolytes rather than proteins contribute most to the osmolality of saliva, the overall secretion rate of solutes (or osmolality) should not be affected by most physiological conditions. To date only one laboratory has reported sIgA relative to osmolality [43].

Because sIgA secretion into saliva indicates the amount of sIgA effectively available on mucosal surfaces, this would appear to be the best way to express sIgA. Few laboratories have reported this measure. A drawback of this method is that it requires the correct assessment of total saliva flow rate without any losses (e.g. due to swallowing). It also requires exact timing of the period of sampling. Poor compliance of subjects adversely affects this measurement.

The present study indicates that sIgA:Alb is the most reliable and relevant way to express changes in sIgA for the following reasons:

- sIgA:Alb results for the diurnal variation, cohort and stress studies have the smallest SDs (and apparent analytical error) and smallest within-subject biological variation;
- sIgA:Alb appears to be the measure least sensitive to changes in saliva flow rate and hydration status; and
- measurement of salivary albumin is technically easier than either osmolality or saliva flow rate and is prone to less error.

4.2 Diurnal variation

The flow rate and composition of unstimulated whole saliva shows a significant circadian rhythm [55] and several authors have reported diurnal variations in sIgA secretion into saliva with early morning values being higher than afternoon values [56, 57, 58]. One explanation for these observations is a relationship between bright light exposure, sIgA secretion and melatonin activity [59]. However, not all laboratories report diurnal variation in sIgA secretion [33] and the results need to be interpreted with caution due to the methodological problems mentioned earlier. The results presented in section 3.1 provide no evidence for diurnal variation, although there was a trend for higher early morning values.

4.3 Treadmill stress tests

4.3.1 Strain experienced

Fifteen men and 5 women completed the stress tests. The mean age of the group was 26.9 years (range 18.1 to 54.5 years) and the mean $\dot{V}O_{2max}$ was 44.3 mL/kg/min for the men (46.63 mL/kg/min for men under 30 years) and 39.1 mL/kg/min for the women (mean age 23.0 years). The aerobic fitness of this group can be described as marginally above the mean expected of healthy sedentary adults (40–45 mL/kg/min for men and 30–35 mL/kg/min for women) [60] and a little higher than the mean value (41.6 mL/min/kg) found for 108 randomly selected, healthy Australian men aged 30 to 39 [61].

The HH test was moderately strenuous for most subjects as evidenced by peak heart rates up to 90% of the subject's age-predicted maximum (Table 6) and peak tympanic temperatures in the range 36.5–39.5°C. Perceived exertion, which correlated well with heart rates, indicated moderate cardiovascular strain during the HH test and low to moderate during the CD test. Several subjects showed distress towards the end of the HH tests and one subject failed to complete the test because of exhaustion. Perceived thermal strain was moderate to high for the HH test in contrast to the CD test where subjects' perception of thermal strain was low.

All subjects maintained satisfactory hydration status by drinking 0.5-2.4 L/h (mean 1.3 /h) of water during the HH test and 0.3-1.4 L/h (mean 0.8L/h) during the CD test. Four subjects who drank 1.3 L/h (HH test), 0.75 L/h (HH test), 0.5 L/h (CD test) and 0.4 L/h (CD test) were clinically hypohydrated (urine SG $\geq$ 1.030) on completion of testing. However, a state of hypohydration for these subjects is not supported by evidence of body mass loss or decreased body water content and could be false positive results of the indirect chemical test used to measure urine SG. Water consumption of 1.3 L/h to maintain euhydration during a moderately strenuous activity in a hot humid environment is consistent with the previous recommendation of 1.5 L/h for arduous activities such as route marching or patrolling in hot/dry environments [62].

4.3.2 Salivary secretory IgA response to stress tests

Acute changes in measures of sIgA were observed in the first hour following both stress tests with increases in sIgA concentration and secretion and a decrease in sIgA:Alb (Figure 2). Datasets for the next 23 hours were less reliable, but indicated a return to baseline values after the CD stress tests (for all sIgA measures). After the HH stress test values for concentration and secretion rate returned to baseline while the ratio measurement, sIgA:Alb, remained suppressed. Gender differences were not detected, but the study was limited by the small number of female subjects.

For the reasons outlined above, the sIgA:Alb measure appeared to be the most reliable measure of sIgA status. This measure indicated a mild decrease in sIgA during the first hour following the CD test with a gradual increase to baseline values by 24 hours. Following the HH test there was an average decline of 50% which had recovered only to an average of 30% of baseline values by 24 hours. The apparently different responses to the two stress tests approached significance ($p = 0.059$).

This study supports the premise that thermal and cardiovascular strain resulting from moderately vigorous physical activity in hot-humid conditions has a suppressive effect on mucosal immunity for at least 24 hours following completion of the activity. Similar physical activity undertaken in cool-dry conditions (i.e. low-moderate heat strain) has a relatively milder and less prolonged suppressive effect on mucosal immunity.

5. Conclusions and Recommendations

1. The large biological variation and index of individuality for measures of sIgA casts doubt on the value of sIgA as a marker of stress. That is, a one-off measurement compared against a population-based reference interval will have little value in predicting risk of ill-health for that individual. However, sIgA may be useful at the individual level to detect trends in mucosal immunity (serial measurements) or as a single measurement compared against the individual's determined homeostatic value.

- *More work is warranted to improve the sIgA assay by the purchase of new instrumentation and implementation of better sample collection and storage methods.*
- *The stability of sIgA to conditions of collection, transport and storage needs to be defined.*
- *Biological variation should be re-determined once new instrumentation and collection procedures are in place.*
- *A reference interval for healthy adults should be determined using a large cross-sectional population.*

2. The pilot study to establish whether or not sIgA measurements were subject to diurnal variation was not conclusive. An improved experimental design would have better statistical power (ie more subjects) and account for possible post-prandial effects.

- *An experiment should be conducted to establish the existence (or not) of a diurnal variation in sIgA secretion into saliva.*

3. The present study presented some evidence that sIgA:Alb was the best (and most practical) measure of sIgA status, but the study was not specifically designed for this purpose.

- *An experiment should be conducted to determine the best measure of sIgA. Such a study should consider variables such as saliva flow rate, saliva water volume, saliva osmolality as well as concentrations of sIgA and albumin.*

4. This study supports the premise that thermal and cardiovascular strain resulting from moderately vigorous physical activity in hot-humid conditions has a suppressive effect on mucosal immunity. Equivocal results from previous similar studies have most likely been due to non-standardisation of sIgA assay methods [43, 63, 64]. However, the possible additive effect of hot environments on exercise-induced suppression of mucosal immunity should be confirmed by follow-up experiments.

- *Further laboratory investigation of the effect of physical activity intensity and differing environmental conditions on mucosal immunity is required.*

5. The long-term effect of post-exercise mucosal immune suppression such as that reported here is not known. Because high incidences of URTI have been reported amongst

deployed military personnel [32, 65], it is reasonable to propose that failure to recover from post-exercise mucosal immune suppression as a result of inappropriate work-rest cycles, might be causal.

- *Further work is required to ascertain whether ADF work-rest cycles for typical military activities, particularly those conducted under the hot conditions of northern and central Australia may have a deleterious effect on immune function.*

6. Salivary secretory IgA is the only immune parameter so far to be directly associated with the appearance of URTI and is considered the most promising marker of individual risk for the development of URTI [66]. To date work conducted by this laboratory has shown that diet restriction (particularly energy restriction), consumption of alcohol, body mass loss, negative emotions and moderate to high thermal strain suppress sIgA:Alb. In two of these studies the incidence of URTI was found to be negatively associated with sIgA:Alb [26, present study]. Hence sIgA appears to be promising as a biomarker of general physiological and psychological stress.

- *Further work is warranted to investigate the practicality of using sIgA as a biomarker of general stress during training and operations, including stress of psychological, nutritional and environmental origin, and that resulting from arduous physical activity.*

7. Under the conditions of this study, tympanic temperature, as measured by Thermoscan IRT 3520, Type 6013, was not a good surrogate for body core temperature. If a second physiological measure of heat strain is needed for safety purposes (to complement heart rate measurement), it is recommended that body core temperature be measured directly, e.g. by rectal thermal probe or by use of 'radio pills'.

6. References

1. Chodirker W.B. & Tomasi T.B. (1963). Gammaglobulins: Quantitative relationships in human serum and nonvascular fluids. *Science,* 142:1080-1081.

2. Gleeson M. (2000). Mucosal immunity and respiratory illness in elite athletes. *Int. J. of Sports Med.,* 21: S33-S43.

3. McKenzie D. C. (1999). Markers of excessive exercise. *Can. J. of App. Phys.,* 24 (1): 6-73.

4. Booth, F.W., Gordon, W.S., Carlson, C.J. & Hamilton, M.T. (2000). Waging war on modern chronic diseases: primary prevention through exercise biology. *J. App. Physiol,* 88:774-787.

5. American College of Sports Medicine. (2000). <u>ACSM's guidelines for exercise testing and prescription</u>, Sixth Edition, Lippincot, Williams & Wilkins, Philadelphia.

6. McDowell, S.L., Chaloa, K., Housh, T.J., Tharp, G.D. & Johnson, G.O. (1991). The effect of exercise intensity and duration of salivary immunoglobulin A. *Eur. J. App. Physiol. Occ. Physiol,* 63:108–111.

7. Tomasi, T.B., Trudeau, F.B., Czerwinski, D. & Erredge, S. (1982). Immune parameters in athletes before and after strenuous exercise. *J. Clin. Immunol.,* 2:173–178.

8. Gleeson, M., McDonald W.A., Pyne, D.B., et al. (2000). Immune status and respiratory illness for elite swimmers during a 12-week training cycle. *Int. J. Sports Med.,* 21:302-307.

9. MacKinnon, L.T., Chick, T.W., van As, A. & Tomasi, T.B. (1989). Decreased secretory immunoglobulins following intense endurance exercise. *Sports Train. Med. Rehabil.,* 1:209-218.

10. MacKinnon, L.T., Ginn, E. & Seymour G.J. (1993). Decreased secretory immunoglobulin A secretion rate after intense interval exercise in elite kayakers. *Eur. J. Appl. Physiol.,* 67:180-184.

11. Muns, G., Leisen, H., Riedel, H. & Bergmann, K-CH. (1989). Influence of long distance running on IgA in nasal secretion and saliva. *Dtsch. Z. Sportsmed.,* 40:63-65.

12. Gleeson, M. (2000). Mucosal immune response and risk of respiratory illness in elite athletes. *Exerc. Immunol. Rev.,* 6:5-42.

13. Douglas D.J. & Hanson, P.G. (1978). Upper respiratory infections in the conditioned athlete. *Med. Sci. Sports Exerc.*,10:55-59.

14. Peters, E.M. & Bateman E.D. (1983). Ultra marathon running and upper respiratory tract infections. *S. Afr. Med.J.*, 64:582-584.

15. Shephard, R.J. (1998). Immune changes induced by exercise in an adverse environment. *Can. J. Physiol. Pharmacol.*, 76: 539-546.

16. Brenner, I.K.M., Shek, P.N. & Shephard, R.J. (1995). Heat exposure and immune function: potential contribution to exercise response. *Exerc. Immunol. Rev.*, 1:49-80.

17. Hoffman-Goetz, L. & Pedersen, B.K. (1994). Exercise and the immune system: a model of the stress response? *Immunology Today*, 15:382-387.

18. Kiecolt-Glaser J.K. & Glaser, R. (1991). Stress and immune functions in humans. In: Ader, R., Felton D.L., Cohen, N. eds. Psychoneuroimmunology. Second Edition, pp849-867, Academic Press, New York.

19. Martin, R.A. & Dobbin, J.P. (1988). Sense of humour, hassles and immunoglobulin A: Evidence for a stress moderating effect of humour. *Int. J. Psychiat. Med.*,18:93-105.

20. Farne, M.A., Boni, P., Corallo, A., Gnugnoli, D. & Sacco, F.L. (1994). Personality variables as moderators between hassles and objective indications of distress (sIgA). *Stress Med.*, 10:15-20.

21. Graham, N.M.H., Bartholomeusz, R.C.A., Taboonpong, N. & La Brooy, J.T. (1988). Does anxiety reduce the secretion rate of secretory igA in saliva? *Med. J. Aust.*, 148:131-133.

22. Jemmott, J.B., Borysenko, J.Z., Borysenko, M., McClelland, D.C., Chapman, R., Meyer, D. & Benson, H. (1983). Academic stress, power motivation, and decrease in salivary secretory immunoglobulin A. *The Lancet*, June:1400-1402.

23. Fairbrother, B., Shippee, R., Kramer, T., Askew, W., Mays, M. et al. (1995) Nutritional and immunological assessment of soldiers during the special forces assessment and selection course. Technical Report T95-22, U.S.Army Research Institute of Environmental Medicine, Natick, MA.

24. Shippee, R., Friedl, K., Kramer, T., Mays, M., Popp, K. et al. (1994). Nutritional and immunological assessment of ranger students with increased caloric intake. Technical Report T95-5, U.S.Army Research Institute of Environmental Medicine, Natick, MA.

25. Booth C., Coad, R., Forbes-Ewan, C., Thomson, G. & Niro, P. (2001). Changes in Nutritional, Immunological, and Psychological Status of Airfield Defence Guards Consuming Australian Combat Rations During a Routine Patrol Exercise. DSTO Research Report, *in press*, Aeronautical and Maritime Research Laboratory, Melbourne, Vic.

26. Carins, J. & Booth, C. (2001) Evaluation of a Simple Immunological Test (sIgA) During the RAAF Survival Course. DSTO Research Report, *in press*, Aeronautical and Maritime Research Laboratory, Melbourne, Vic.

27. Cotter, J.D., Roberts, W.S., Amos, D., Lau, W-M. & Prigg S.K. (2000). Soldier Performance and Heat Strain During Evaluation of a Combat Fitness Assessment in Northern Australia. DSTO-TR-1023, Aeronautical and Maritime Research Laboratory, Melbourne, Vic.

28. Chicharro, J.L., Lopez-Mojares, L.M., Lucía, A., Pérez, M., Alvarez, J., Labanda, P., Calvo, F. & Vaquero, A.F. (1998). Overtraining parameters in special military units. *Aviat. Space Environ. Med.*, 69:562-568.

29. Fry, J., Morton, N., Crawford, C. & Keast D. (1992). Cell numbers and in vivo responses of leukocytes and lymphocyte subpopulations following maximal exercise and interval training sessions of different intensities. *Eur J. Appl. Physiol.*, 64:218-217.

30. Aoyagi, Y., McLellan, T.M. & Shephard, R.J. (1998). Effects of endurance training and heat acclimation on psychological strain in exercising men wearing protective clothing. *Ergonomics*, 41:328-357.

31. Shephard, R.J. & Shek, P.N. (1999). Immune dysfunction as a factor in heat illness. *Crit. Revs Immunol.*, 19:285-302.

32. Department of Defence (2000). *Australian Defence Force Health Status Report*. First Edition. Defence Publishing Service, Canberra ACT.

33. Gleeson, M., Cripps, A.W., Clancy, R.L. & Geraghty, S.B. (1990). The variability of immunoglobulins and albumin in saliva of normal and IgA-deficient adults. In T.T. MacDonald (Ed.), Proceedings of the 5th International Congress on Mucosal Immunity: Advances in mucosal immunity, pp. 500-501, Kluwer Academic, Lancaster.

34. Nieman, D.C. (1994). Exercise, upper respiratory tract infection, and the immune system. *Med. Sci. Sports Exerc.*, 26:128-139.

35. Nieman, D.C. (1997). Exercise immunology: Practical applications. *International J. Sports Med.*, 18(Suppl. 1):91–100.

36. Pyne, D. (1999). Balancing training to keep your athletes healthy. *Sports Coach*, 22(3):20–22.

37. Pyne, D.B., Gleeson, M., McDonald, W.A., Clancy, R.L., Perry, C. & Fricker P.A. (2000). Training strategies to maintain immunocompetence in athletes. *Int. J. Sports Med.*, 21 (suppl. 1):51–60.

38. Walsh, N.P., Blannin, A.K., Clark, A.M., Cook, L., Robson, P.J. & Gleeson, M. (1999). The effects of high-intensity intermittent exercise on saliva IgA, total protein and α-amylase. *J. Sports Sci.*, 17:129-134.

39. Amoateng-Adjepong, Y., Mundo, J.D. & Manthous, C.A. (1999). Accuracy of an infrared tympanic thermometer. *Chest*, 115:1002-1005.

40. Hansen, R.D., Amos, D. & Leake B. (1996). Infrared tympanic temperature as a predictor of rectal temperature in warm and hot conditions. *Aviat. Space Env. Med.*, 67:1048-1052.

41. Jakobsson, J., Nilsson, A. & Carlsson, L. (1992). Core temperature measured in the auricular canal: comparison between four different tympanic thermometers. *Acta Anaesthesiologica Scand.*, 36:819-824.

42. Leger, L.A. & Gadoury, C. (1989). Validity of the 20 m shuttle run test with 1 min stages to predict $\dot{V}O_{2max}$ in adults. *Can. J. Sport Sci.*, 14(1):21-6.

43. Blannin, A.K., Robson, P.J., Walsh, N.P., Clark, A.M., Glennon, L. & Gleeson, M. (1998). The effect of exercising to exhaustion at different intensities on saliva immunoglobulin A, protein and electrolyte secretion. *Int. J. Sports Med.*, 19:547-452.

44. Chaseling, D. (1994). A Pilot study in the Design of Methods for Determination of Magnesium Status. Queensland University of Technology Masters thesis.

45. Brewer, J., Ramsbottom, R. & Williams C. (1988). Multistage fitness test. A progressive shuttle-run test for the prediction of maximum oxygen uptake. Belconnen, ACT, Australian Coaching Council, Australia.

46. Sutton, J.R. (1994). Physiological and clinical consequences of exercise in heat and humidity. In M. Harries, C. Williams, W.D. Stanish & L.J. Micheli (Eds.), Oxford textbook of sports medicine, pp. 231-237, Oxford University Press, London.

47. Sato, K.T., Kane, N.L., Soos, G., Gisolfi, C.V., Kondo, N. & Sato, K. (1996). Reexamination of tympanic membrane temperature as a core temperature. *J. App. Physiol.*, 80:1233-1239.

48. Defence Nutrition Research Centre (2001). Bioimpedance analysis. DNRC Method ID 897/1424, version 1.2.

49. Fraser, C.G. (1994). Data on biological variation: Essential prerequisites for introducing new procedures? *Clin. Chem.*, 40:1671-1673.

50. Fraser, C.G. & Harris, E.K. (1989). Generation and application of data on biological variation in clinical chemistry. *Cit. Revs. Clin. Lab. Sci.*, 27:409-430.

51. Solberg, H.E. (1994). Establishment and use of reference values. In C. A. Burtis & E.R. Ashwood (Eds.), Tietz textbook of clinical chemistry, Second Edition, pp. 454-484, W.B. Saunders, Sydney.

52. Stone, A.A., Cox, D.S., Valdimarsdottir, H., Jandorf, L. & Neale, J.M. (1987). Evidence that secretory IgA antibody is associated with daily mood. *J. Pers. Soc. Psych.*, 52:988-993.

53. Mackinnon, L.T. (1999). Exercise and humoral immunity: Immunoglobulin, antibody, and mucosal immunity. In L.T. Mackinnon (Ed.), Advances in Exercise Immunology, pp. 159–200, Human Kinetics, Champaign, IL.

54. Walmsley R.N. & White G.H. (1994). A Guide to Diagnostic Clinical Chemistry, pp270-271, Blackwell Scientific Publications, Melbourne, VIC.

55. Dawes, C. (1972). Circadian rhythms in human salivary flow rate and composition. *J. Physiol.*, 220:529-545.

56. Miletic, I.D., Schiffman, S.S., Miletic, V.D. & Sattely-Miller, E.A. (1996). Salivary IgA secretion rate in young and elderly persons. *Physiol. Behav.*, 60:243-248.

57. Richter, J., Král, V., Žukov, I., Subrt, P. & Rahm, J. (1980). Circadian changes of the SIgA, lysozyme, albumin and copper content of saliva. *Czech. Med.*, 3:249-254.

58. Hucklebridge, F., Clow, A. & Evans, P.D. (1998). The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. *Int. J. Psychophysiol.*, 31:69-76.

59. Park, S.J. & Tokura, H. (1999). Bright light exposure during the daytime affects circadian rhythms of urinary melatonin and salivary immunoglobulin A. *Chronobiol. Int.*, 16:359-371.

60. McArdle, W.D., Katch, F.I. & Katch, V.L. (1991). Exercise Physiology: Energy, Nutrition and Human performance. Third Edition, p212. Lea & Febiger, Philadelphia, USA.

61. DASETT (1992). Pilot Survey of the Fitness of Australians. Department of the Arts, Sport, Environment, Territories and Tourism. Catalogue No. 9218272. AGPS, Canberra.

62. Lau, W-M., Roberts, W. & Forbes-Ewan, C. (1999). Physiological Performance of Soldiers Conducting Long Range Surveillance and Reconnaissance in Hot, Dry Environments. DSTO-TR-0787, Aeronautical and Maritime Research Laboratory, Melbourne, Vic.

63. Hübner-Woźniak, E., Lutoslawska, G. & Sendecki, W. (1998). Effect of training volume on the levels of salivary immunoglobulin A in wrestlers. *Biol. Sport*, 15:129-131.

64. Buckwalter, J.B., Moore, S.E., Bhunia, A.K. & Fort, I.L. (1996). The acute response of salivary immunoglobulin A to moderate exercise in women (Abstract). *Med. Sci. Sports Exerc.*, 28 (Suppl.):92.

65. Linenger, J.M, Flinn, S., Thomas, B., et al. (1993). Musculoskeletal and medical morbidity associated with rigorous physical training. *Clin. J. Sport Med.*, 3:229-234.

66. Mackinnon, L.T. (2000). Overtraining effects on immunity and performance in athletes. *Immunol. Cell Biol.*, 78:502-509.

Appendix 1: Physical Activity Readiness Questionnaire

Name: Service No: Date:

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For most people, physical activity should not present any risk or hazard. The PARQ has been designed to identify the small number of adults for whom physical activity might be inappropriate or those who should have medical advice concerning the type of activity most suitable.

Please circle the appropriate answer

1.	Has a doctor ever said you have heart trouble?	YES	NO
2.	Do you frequently suffer from pains in the chest?	YES	NO
3.	Do you often feel faint or have spells of severe dizziness?	YES	NO
4.	Has a doctor ever said your blood pressure was too high?	YES	NO
5.	Has a doctor ever told you that you have a bone or joint problem such as arthritis that has been aggravated by exercise, or may be made worse with exercise?	YES	NO
6.	Is there any good physical reason not mentioned here why you shouldn't perform exercise even if you wanted to (asthma, medications, etc.)?	YES	NO
7.	Are you over 65 and not accustomed to physical exercise?	YES	NO

If a person answers "YES" to any of these questions, vigorous exercise or exercise testing should be postponed. Medical clearance may be required.

Resting Blood Pressure   ...../......   Investigator.............................

DNRC ID.............................   DATE.................*(office use, only)*

Appendix 2: Consent and Information Forms

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INFORMED CONSENT FORM

**LABORATORY-BASED EXERCISE &
ENVIRONMENT STRESS STUDY**

Task No: ARM 01/067

I,.. give my consent to
participate in the study mentioned above on the following basis:

◆ I have had explained to me the aim of this research, how it will be conducted and my
role in it. I am happy to participate.

◆ I understand that I have agreed to have my health and dietary status screened for
my participation in an exercise and environmental stress study. I will also have my
maximum aerobic capacity measured.

◆ I understand that I have been asked to provide saliva samples routinely for a period of
eight weeks so that my usual range of saliva immunoglobulin A (IgA) levels can be
determined. Because IgA levels can be affected by my personality type, mood state,
medical condition and menstrual cycle I will be asked to complete some questionnaires
concerning these factors.

◆ I understand that I have been asked to complete a modified Combat Fitness
Assessment test within an environmental chamber on two occasions – once under hot
humid conditions and once under cool, dry conditions. The effects of these tests on my
immune system, psychological state and body hydration will be determined by
measurement of IgA in my saliva, completion of simple psychology questionnaires and
by measurement of my body composition (body mass and bioelectrical impedance).

♦ Throughout the tests I will have my heart function and body temperature monitored. An electrocardiograph will be used to monitor my heart function and an infra red thermometer will be used to measure my ear temperature. The testing will be supervised by personnel with first-aid qualifications. The test will be stopped should my heart rate exceeds a safe maximal limit for three consecutive minutes or should my body temperature reach 39.5°C or at the discretion of the medical monitor or the investigator. I can stop the test at any time.

♦ I understand that I am participating in this study in a voluntary capacity and can withdraw at any time without detriment to my career or studies and without compromise to my medical care.

I am co-operating in this study on condition that:
- the information I provide will be kept confidential
- the information will only be used for this project
- the testing will be conducted whilst I am 'on duty'
- the results will be made available to me at my request and
- any published reports of this study will preserve my anonymity

♦ I have been given a copy of the Australian Defence Medical Ethics Committee's Guidelines for Volunteers, an information sheet and this form, signed by me and by the principal researcher, Dr Christine Booth.

INFORMATION SHEET
LABORATORY-BASED EXERCISE & ENVIRONMENT STRESS STUDY

Task No: ARM 01/067

Investigators:
Mr Paul Pacqué, University of Tasmania
Dr Christine Booth, Defence Nutrition Research Centre
Mr Dan Dwyer, University of Tasmania

Overtraining, or overwork, is defined as a situation of prolonged fatigue and decreased performance following a period of heavy exercise with incomplete recovery. Personality traits can influence how people adapt to chronic stress.

The level of an immunoglobulin, secretory IgA, in saliva is being tested as a possible predictor of overtraining/work. We believe secretory IgA might be a good predictor of overtraining, because chronic stresses such as under-nutrition, heavy exercise and emotional stress have been shown to result in decreased secretion of IgA into the saliva.

Overtraining, which results in frequent colds, sore throats and sinus infections amongst triathletes and elite swimmers, has been linked with decreased salivary secretory IgA (sIgA). Furthermore, the saliva test is simple to perform and non-invasive.

Should sIgA prove to be a predictive test of overtraining, then the next step would be the development of a robust and cheap method of analysis suitable for direct measurement of IgA in the field. Such a simple test might be used in a military setting by commanding officers to predict overwork particularly in training situations.

You are being asked to:
- Undergo a screening test, which consists of a health & diet assessment (by questionnaire), body mass index measurement (height and weight) and, if you are a civilian, the Army's Basic Fitness Assessment (BFA);
- Complete a personality traits questionnaire and (for women) complete a menstrual cycle questionnaire;
- Donate saliva samples and complete a medical check list for minor illness and/or injury, three times each week for 8 weeks;
- Complete a psychology test for mood once each week for eight weeks;
- Have your peak aerobic capacity measured (by a shuttle run test) and
- Complete two exercise sessions in an environmental chamber once under hot, humid conditions and once under cool dry conditions.
  1. The study will be administered by University of Tasmania staff through Patterson Barracks (for ARA volunteers) and through the School of Human Movement Studies (for civilian volunteers).

  2. Because the exercise and environmental stress study requires a reasonable physical fitness, you will be asked about any possible health problems. You will also be asked to record the foods you eat and any dietary supplements or medications you may be taking and to have your body mass index checked [weight/(height)^2]. You will receive the results of the dietary assessment with any appropriate dietetic recommendations.

  3. YOU MUST NOT VOLUNTEER FOR THIS STUDY IF YOU ARE PREGNANT OR IF YOU ARE INTENDING TO BECOME PREGNANT DURING THE NEXT FOUR MONTHS.

  4. If you have a healthy diet and no health problems you will be invited to participate in the study. You will now be asked to complete a personality traits questionnaire. You will receive the results of this questionnaire and should you wish, a registered psychologist will be able to give you further information about your results.

  5. In order to determine your usual saliva IgA level you will be asked to provide an early morning (before breakfast) saliva sample on three mornings each week for eight weeks. [You will be given instruction on how to collect and label the sample and how to store them] Because personality mood and minor health problems can

influence secretion of IgA, you will be asked to complete a simple medical checklist each time you collect a saliva sample and each week, when you return your samples to Paterson Barracks/ Uni of Tas, you will be asked to complete a psychology questionnaire which records your mood profile for the past week. You will also have your weight recorded on a weekly basis.

6. Exercise and environmental testing will be supervised by personnel with first-aid qualifications.

7. After you have enrolled in the study, you will be asked to present at the Human Performance Laboratory, University of Tasmania to perform a shuttle run test for the purpose of determining your maximal oxygen uptake ($VO_{2 \, max}$). This test involves running back and forth (20m) across the gymnasium to a (decreasing) timed cadence till you feel you can't continue. During this visit the scientific team will show you the environmental chamber and the physiological testing equipment and explain the next round of tests to you.

8. You will be asked to present at the Human Performance Laboratory (Uni of Tas) Environmental Chamber on two further occasions to complete a modified Combat Fitness Assessment (CFA) to be conducted in an environmental chamber. The environmental conditions will be set at a "hot" or a "cool" setting (hot: 28°C and 80% relative humidity, cool: 15°C and ambient relative humidity) and will be maintained throughout the entire test to within ± 2°C and ± 5% relative humidity. The CFA will be performed in Patrol Order (20 kg total weight) over 10 km and should be completed in 120 minutes. Chilled water will be provided. You will be able to shower and change into fresh clothes on completion of the test.

9. During the CFA your safety will be carefully monitored by a continuous measurement of your heart function (electrocardiograph) and by regular measurement of you inner ear temperature. A trained first aider will be in attendance. The test will be stopped at the discretion of the first aid attendant or the investigator. You may stop the test at any time and likewise are free to withdraw from the study at any time.

10. The effects of the CFA on your immune system and physiological state will be determined by some simple tests. Firstly you will be asked to give saliva samples at timed intervals before and after the test. Secondly you will be asked to complete some simple questionnaires which gauge your anxiety and level of discomfort and thirdly your hydration level will be determined by recording your body composition. Body composition will be determined by measurement of your weight (in under wear - bike shorts and crop top acceptable) and bioelectric impedance analysis (BIA). BIA involves passing a minute electric current (so weak that it you cannot detect it) through your body. By measuring the electrical impedance of your body we can estimate how much body water you have and thereby calculate your body fat mass.

11. Benefits to you for your participation in this study include body composition and diet assessment and measurement of peak aerobic capacity. Furthermore, professional advice regarding diet and the psychological testing will be available at no cost.

12. <u>Your involvement in this study is voluntary</u>. You are free to withdraw from this study at any time without detriment to your career or studies. The information collected will be kept confidential and nothing will be published which will identify individual participants. The information will only be used for this study.

Should you have any concerns or questions regarding the conduct of this study, please contact:

Executive Secretary of the Australian Defence Human Research Ethics Committee
CP4-6-45 Department of Defence
CANBERRA ACT 2600
Phone: 02 6266 3925; DNATS 8663925 Fax: 02 6266 4982; DNATS 8664982
e-mail: <u>hlthpol@bigfoot.com</u>
OR
Dr Janet Vial,
University Human Research Ethics Committee
University of Tasmania
Phone: 6226 4842 or Chris Hooper, phone: 6226 2763

For questions or further information about this research, contact the investigators

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Appendix 3: Physical Condition Report

Name:........................   Service No:........................ Date:........................

✂ --

Physical condition report (Self Assessment)

Please tick any physical conditions that you have TODAY

Condition	Yes	No	Office use
A cough, cold, runny nose or sore throat			URTI
Skin complaints (rashes, sores)			FI
Any insect bites			IB
A headache			HD
An allergic reaction			AR
Stomach upset, vomiting, nausea			GIT
A fracture, dislocation or muscle strain			F/D
Cuts, bruises			TRA
Sunburn			SB
Any other physical symptom (describe):			OTH

DNRC ID........................... DATE........................ *(office use, only)*

Appendix 4: Questionnaires of Perceived Strain

<u>**THERMAL SENSATION SCALE**</u>   <u>**THERMAL DISCOMFORT SCALE**</u>

THERMAL SENSATION SCALE	THERMAL DISCOMFORT SCALE
1 unbearably cold	1 comfortable
2 extremely cold	2 slightly uncomfortable
3 very cold	3 uncomfortable
4 cold	4 very uncomfortable
5 cold	5 extremely uncomfortable
6 slightly cold	<u>**PERCEIVED EXERTION SCALE**</u>
7 neutral	6
8 slightly warm	7 very, very light
9 hot	8
10 very hot	9 very light
11 extremely hot	10
12 unbearably hot	11 fairly light
	12
	13 somewhat hard
	14
	15 hard
	16
	17 very hard
	18
	19 very, very hard

Appendix 5: Menstrual Cycle Questionnaire

Name: Service No: Date:

✂---

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Your body temperature rises by approximately 0.5°C when you ovulate, and it remains higher during the next fortnight or so until your period begins. Therefore, it is possible that your body temperature will be different in the two CFA trials simply because of your menstrual cycle. If so, this could incorrectly make it appear that your body is coping worse (if a higher temperature) or better (if a lower temperature) in the second CFA due to your PT program. Unlike many research projects using female participants, we are not trying to plan the two CFAs to occur when you are in the same phase of your menstrual cycle because it is not practical (eg. no two cycles are the same), and it does not reflect reality. However, if your body temperature responses or physical performance seems unusual compared with those of other female participants, it would be useful for us to be able to estimate which half of the menstrual cycle (if either) you were in during each CFA. Because of this, we would appreciate it if you answered the following questions. Remember that any information you provide is strictly confidential and will only be used if necessary, and only by a female member of the scientific team. Even so, you are not obligated to provide any information that you do not wish to, and you should not feel awkward if you choose not to.

1. Are your periods regular (ie. spaced at regular intervals)? Yes / No

2. If Yes, approximately how long is your cycle (eg. 28 days)?

3. Approximately how many days has it been since the beginning of your last period?

Thank you.

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of Immune Suppression Following the
Combat Fitness Assessment

Paul Pacqué, Christine Booth and Dan Dwyer

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19. ABSTRACT

SIgA is a potential biomarker for stress. The usual day-to-day and within day variation in sIgA amongst a group of healthy Army reservists was estimated and the acute response of sIgA to moderate intensity exercise (Combat Fitness Assessment) undertaken in both cool-dry and hot-humid conditions was determined. The results indicate that thermal and cardiovascular strain resulting from moderate intensity exercise in hot-humid conditions suppresses sIgA for at least 24 hours post-exercise. Salivary sIgA exhibits a wide biological variation which casts some doubt on its usefulness as a biomarker, however because sIgA has been shown to be sensitive to dietary restriction, alcohol consumption, loss of body mass, fatigue and negative emotions in previous studies and now heat-induced cardiovascular strain, further work is warranted to develop this biomarker.

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