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The Physiological and Performance Effects of Simulated Race Rowing on Female Lightweight Rowers in a Euhydrated and Hypohydrated State

by
Michael Andrew Penkman

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Masters of Science

Department of Physical Education and Recreation

Edmonton, Alberta
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ABSTRACT

The purpose of this study was to determine the consequences of weight restriction using dehydration, followed by the performance of a simulated 2000m rowing race on immune cell numbers and function, core temperature and performance. Seven healthy females between the ages of 18- 28 performed a simulated rowing performance in both a euhydrated and hypohydrated state. In the rehydrated trial (RT) subjects completed a 2000m simulated rowing test following a 24 hr period of fluid restriction and light exercise to decrease their body mass and a 2 hour rehydration period. All subjects also completed a euhydrated trial where no body mass was lost. The order of treatments were randomized. Time to complete the 2000m distance, tympanic temperatures, complete blood counts, immunophenotyping and functional analysis of lymphocytes and neutrophils were determined. There was no difference in time to complete the 2000m distance between the two trials following a body mass decrease of 3.33%. The rehydration period allowed all of the body mass to be regained, but a significant elevation in tympanic temperatures was observed throughout the RT ($p<0.05$). The immune response was similar between the two trials with the exception of a larger post-test leukocytosis in the RT consisting of a more rapid rise in neutrophils and a higher concentration of $CD16^+$ lymphocytes throughout the trial ($p<0.05$). In contrast to previous results observed on male rowers (Nielsen et al., 1996; 1998) a decrease in Con A stimulated lymphocyte proliferation (represented by stimulation index) was observed post-test and following one hour of recovery and the concentration of $CD4^+$ and $CD4^+25^+$ lymphocytes were lower than pre-test values during recovery ($p<0.05$). Neutrophil

oxidative burst activity was lower post-test at the 10 min time point of the assay ($p<0.05$). These results suggest that while a 3.33% decrease in body mass through fluid restriction and sweating did not lead to a level of dehydration that was sufficient to impair performance following a 2 hr rehydration, it did lead to an increase in the heat stress in lightweight female rowers. Furthermore, the immune response observed in these individuals suggest that the intense effort, large muscle mass rowing activity has a greater impact the immune system of lightweight female rowers than men.

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LIST OF SYMBOLS, NOMENCLATURE OR ABBREVIATIONS

MAb	Monoclonal Antibody
FITC	Fluorescein Isothiocyanate
PE	Phycoerythrin
B	Biotin (conjugated with streptavidin)
ET	Euhydrated Trial
RT	Rehydrated Trial (following a 24 hr dehydration protocol)
ANOVA	Analysis of Variance

CHAPTER 1

INTRODUCTION

1.1 Introduction

Rowing is a sport in which competitors perform an all-out effort to cover a distance of 2000m in as short a time as possible. A typical race duration is in the order of 5.5-7.5 minutes depending on the size of the boat and the crew. Traditionally rowing was a sport in which only men competed, but in recent years there has been an increase in participation in the sport by women to a level equal to that of men. In 1980, the category of lightweight became an international rowing event to allow rowers of smaller mass to compete against those of similar size, and in 1996 lightweight rowing became part of the Olympic games.

The advent of the lightweight category moved rowing into the world of weight restrictive sports and with it, the problems associated with administration of the weight division and how to distinguish between a heavyweight and a lightweight rower. The maximum allowable weight for a female rower in the lightweight category is 59.0 kg or 130 lbs, with a boat average of 56.8 kg or 125 lbs. The desire to be competitive in rowers that are too heavy to be in the lightweight category has lead to a practice known as "making weight", or losing weight through a combination of dieting, fluid restriction and sweating over the days prior to the race in order to compete in the lighter category. The athletes often do this without regard for any health risks, believing that they will be just as fast and more competitive at the lighter weight.

International Rowing (Federation Internationale des Societes d'Aviron, FISA) rules require the athlete to weigh in two hours before the official start time of the race, leaving the athletes with typically about 1.5 hours before they will be called to launch their boat onto the water. This time period has been shown to be insufficient for those who used dehydration as a weight restriction technique to return their body fluid to euhydrated levels (Burge, Carey & Payne, 1993). The result is that the rower may be competing in a hypohydrated state. This short time between weigh in and race time was

meant to discourage weight restriction, but many rowers especially at the club and provincial level still practice the same weight restriction procedures.

Medical practitioners and professional groups have recognized the risks of weight restriction techniques in sports such as wrestling. Several research studies have been published in the area of wrestling and dehydration effects on performance (Hansen, 1978; Houston, Marrin, Green & Thomson, 1981; Horswill, 1991; Tipton, 1982). The American College of Sports Medicine (ACSM) published a position stand on weight loss in wrestlers in 1976, later revised in 1996. This position stand states that the primary performance problems resulting from the practice of "making weight" are a potential decreased anaerobic power (conflicting research), reduced endurance capacity, reduced muscle strength, decreased immune function and impaired thermoregulation. In addition, further problems may arise in terms of hormonal status, protein nutritional status, impaired growth and development, psychological state and other health difficulties.

This research has raised concerns for the competitive performance and health problems facing rowers who are trying to make weight; several differences however, make rowing unique. The more intense aerobic power effort in rowing stresses the physiological aspects of the body differently. Therefore, the overall impact of dehydration may also be different. Another issue is that the weigh in for wrestling is completed the previous night resulting in a much longer time period to rehydrate. Wrestling is also a male dominated sport, but has recently begun to include women, while currently in rowing the participation of women is now exceeding that of men.

Research relating to the practice of making weight in rowing has been limited to that which showed weight restriction to be detrimental to the performance of a six minute all-out rowing test by elite lightweight males (Burge et al., 1993), but the effects of the weight reduction practice beyond that of performance, or on female rowers have not been studied. This is in stark contrast to the state in the sport of wrestling where the issues of weight reduction is frequently in the news and many comprehensive studies have been conducted on this issue.

The potential for heat illness in those athletes who use dehydration as a means to

reduce weight over a short time period has been described as their biggest health concern (ACSM,1996). The changes in core body temperature have been characterised for several exercise tasks, but the extent to which high intensity rowing exercise challenges the thermoregulatory system has not been investigated. This information is important to those athletes who may consider using dehydration as a means to make weight, especially when the competitive events are often performed in hot, humid environments.

Recently, there has been an increase in the volume of research investigating the effects of exercise on the immune system. It has been suggested that moderate exercise training may improve resistance to primary infection (MacKinnon, 1992), while it has become recognized that acute bouts of long duration exercise to fatigue can be immunosuppressive (Hoffman-Goetsz & Pedersen, 1994). Studies have used a variety of exercise modes in examining the effect on the immune system, however, the majority have used running and cycling. While most of these studies have been performed using acute bouts of moderately high intensity exercise to fatigue, less have looked at the effect of an acute intense performance at near maximal oxygen consumption (VO_2max) intensities. One recent study has examined the effects of a maximal rowing trial on the immune system and showed no evidence of immunosuppression in heavyweight men (Nielsen, Secher, Kappel, Hanel & Pedersen, 1996).

The mechanism by which the enhanced susceptibility to infection is thought to occur has been termed the open window hypothesis (Pedersen and Ullum, 1994). The suggestion is that the exercise-induced drop in the functional status of the immune system occurs for a period of time during recovery which opens the "window" for invading pathogens. Stress in general, such as psychological stress and trauma have also been shown have immunopotentiating effects. These researchers suggest that in this sense, exercise should be considered a subset of stress immunology. It is the potential additive effect of the psychological and physiological stress of making weight in combination with the exercise stress that the lightweight rower must endure, that makes this a unique group for study. In addition, several studies have shown evidence that the immune system of the female is different in its distribution of immune cells and activities than that of the

male (Giglio, Imro, Filaci, Scudeletti, Puppo, De Cecco, Individeri and Costantini, 1994).

Research has shown the impact of dieting on the immune system in overweight subjects (Kelley, Daudu, Branch, Johnson, Taylor & Mackey 1994; Field, Gougeon & Marliss, 1991) and in athletic women (Kono, Kitao, Matsuda, Haga, Fukushima & Kashiwagi, 1988) with both situations showing immunosuppressive action of weight loss. Shephard and Shek (1995) suggested that there may be a concentration of immune problems in sports with substantial weight loss and that athletes in these types of sport warrant further study. These studies involved caloric restriction, but dehydration alone has been suggested to induce changes in the immune system (Greenleaf, Jackson & Lawless, 1995).

There has been some epidemiological evidence that rowers may have an increased incidence of illness, to a level equal to that of long distance runners and elevated above that of controls (Castell, Poortmans & Newsholme, 1996). The link between an observed impairment of the immune system *in vitro*, and a clinically relevant impact on the susceptibility to illness has been a difficult one to determine for exercise immunologists. The long distance endurance runner seems to be the only case with solid laboratory evidence for an impaired immune system and good epidemiological evidence for increased risk of illness (Peters & Bateman, 1983). The data on female lightweight rowers has been limited, and the stress that these rowers undergo using weight restriction procedures warrants investigation. The potential negative immunological, thermoregulatory, performance and possible health consequences resulting from dehydration practices followed by female lightweight rowers is important research. Furthermore, the effect of the performance of a high intensity rowing simulation in a hypohydrated state on immunological parameters and thermoregulatory responses is an area which is yet to be studied.

1.2 Statement of the Problem

The problem upon which the proposed study is based seeks to determine the potential immunological, thermoregulatory and performance consequences for a female lightweight rower as a result of performing a simulated rowing performance of 2000m in a hypohydrated state.

1.3 Research Hypotheses

The performance of the 2000m simulated rowing test in a hypohydrated state will result in a decreased performance when compared to a performance in a euhydrated state. The 2000m performance test in the hypohydrated state will also result in a significantly lower immune and higher thermoregulatory response indicative of immunosuppression and compromised thermoregulation.

1.4 Purpose

The practice of making weight has been shown to be detrimental to the performance of a six minute all-out rowing test by elite lightweight males (Burge et al, 1993), but the effects of the practice beyond that of performance, or on female athletes have not been studied. Therefore, the purpose of this study is to investigate the consequences of weight restriction using dehydration, followed by the performance of a simulated 2000m rowing race on immune cell numbers and function, core temperature and performance.

1.5 Definitions

T Cell: a heterogeneous population of lymphocytes comprising helper/inducer T cells and cytotoxic/suppressor T cells.

B Cell: a type of lymphocyte capable of producing antibody. B cells differentiate into plasma cells.

Natural Killer cell (NK): a large granular lymphocyte that can mediate cytolytic reactions against a variety of neoplastic and virally infected cells

Neutrophil: Neutrophils make up 55-65% of blood leukocytes, and these phagocytic cells have an important role in non specific or innate immunity. Neutrophils are part of the first line of defence to infectious agents and are also involved in muscle tissue inflammation response to exercise induced injury

Euhydration: a term used to describe a state of hydration where the body is at its normal weight (established by baseline weigh-ins over three days) and content of total body water.

Hypohydration: a term used to describe a state of hydration where the body is relatively underweight, and total body water is decreased. A 3% decrease in body weight by dehydration has been shown to lead to physiological changes (Allen, Smith &Miller, 1997). This can lead to a contracted blood volume.

Dehydration: a term used to describe the transition from a euhydrated to hypohydrated state.

"Make Weight": a practice performed by athletes in sports with weight categories such as boxing, wrestling, or rowing in which athletes perform light exercise and restrict food and fluid in an effort to decrease weight over a short period of time.

VO_2max: maximum oxygen consumption, expressed as a volume of oxygen consumed per minute. In % of max terms it can be used as an indicator of exercise intensity.

Chronic exercise: referring to a repeated training stimulus over an extended period of weeks, months or years

Acute exercise: referring to a single or repeated bout of exercise in a single training session

1.6 Assumptions and Limitations

Two significant assumptions have been made in the proposed research project. First, it is assumed that the athletes will be capable of performing the 2000m simulated rowing test to the best of their ability twice within three days, and no significant difference will arise in performance time due to training status or fatigue as the athletes will be asked to refrain from any high intensity training during the testing week. The

second assumption is that the athletes will follow a similar pattern of fluid restriction and sweating to achieve the prescribed amount of weight loss and that they will not be able to fully rehydrate themselves during the rehydration period.

There are several limitations to this study. The immune system is exceedingly complex and the ability to isolate specific responses of the immune system as a result of the experimental design and extrapolate an overall effect is difficult due to the many possible indicators of immune system function. The limitation of a possible interaction between immune factors and the cyclic variation in reproductive hormones leading to the observed differences in immune response cannot be ruled out. The interaction between the immune and endocrine systems has received some attention in the literature but to this point research on the influence of varying levels of sex hormones on the immune response to exercise has not been well studied. This limitation is acknowledged, and the menstrual status of each subject will be assessed by questionnaire. A further question is the ability to determine at what level of immunological suppression do the results show a significant health risk to the subject. This question has not been adequately examined in the literature beyond the suggestion that immunosuppression seems to correlate with increased incidence of infection (Smith, 1995; Hoffman-Goetsz & Pedersen, 1994). The major immunological limitation is that we are only sampling immune cells from peripheral blood. While this is the most common source of immune cells in the literature, the information that can be inferred to immune cells in other tissues, such as the lymphoid tissues, skin, spleen and others where the actual immune response takes place, is questionable (Field, 1996). A final limitation to the study is that the sample is limited to varsity level female lightweight athletes, therefore generalizing of the results to elite female or male athletes will not be possible.

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CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

In this study the principle aims were to determine the immunological, thermoregulatory and performance changes occurring with performance of a rowing test in female subjects at two different levels of hydration. The athletes' performance and thermoregulatory response will be monitored and blood sampled at various time points to determine the immunological response. The review of literature is divided into ten subsections related to this topic: (a) Overview of the Immune System, (b) Exercise and the Immune System, (c) Gender Differences in the Immune Response, (d) Weight Loss and the Immune System, (e) Clinical Significance of Possible Immunosuppression, (f) Consequences of Weight Reduction via Hypohydration, (g) The Physiology of Rowing, (h) Effects of Rowing on the Immune System, (I) Clinical Incidence of Illness in Rowers, (j) Rowing Specific Performance Effects of Weight Loss.

2.2 Overview of the Immune System

The immune system of the human body is a exceedingly complicated and diverse network of cellular and soluble (humoral) components that interact to allow a coordinated quick responding system of protection against invading pathogens. This system can be divided into two subsections based upon function. The innate (natural or non-specific) and the adaptive (specific).

The adaptive side is responsible for the immune systems ability to increase responsiveness to subsequent infections by the same pathogen and is also responsible for distinguishing self from nonself. This subsection of the immune system can be separated into a humoral and cell mediated branch. The cell mediated immunity consists of T and B cells (lymphocytes) of several different subtypes. The humoral branch involves

antibodies and memory. The adaptive system allows improved immune responsiveness with subsequent exposure through the action of lymphocytes and macrophages that inactivate and destroy microorganisms by several mechanisms and memory B cells from the first exposure that increase the responsiveness of these cells. This system is characterized by specificity to the infectious agent and is the basis for the immunization to prevent disease.

The innate half of the immune system utilizes three mechanisms to prevent infection. Physical barriers such as skin, epithelial cells and mucus provide a first line of defence; chemical barriers create an inhospitable environment; and immune cells function to recognize, phagocytize and kill invading microorganisms. The cells of this arm of the immune system consist of phagocytic cells such as macrophages, neutrophils, eosinophils, and natural killer cells while the chemical barriers are complement and acute phase proteins, lysozymes, pH of body fluids and other secretions. This branch of the immune system is not affected by chronic exposure of a prior infection, but is influenced by the adaptive system to improve overall responsiveness of the entire immune system. (Introduction to immune system taken from MacKinnon, 1992 and Smith, 1995)

2.3 Exercise and The Immune System

Historically, the relationship between exercise, immunity and infection was first addressed by Larabee (1902) as large increases in blood neutrophils were documented in four athletes at the 1901 Boston Marathon. It was suggested that these changes parallelled that seen in diseased conditions (ie. inflammatory type leukocytosis). When Baetjer (1932) reviewed exercise and infection, he stated that although a common view existed that muscular fatigue lowers resistance to infection, little experimental work had been done to test this belief scientifically. Since then, the quantity of research performed on exercise and immunology has increased dramatically. Presently, health professionals, coaches and athletes tend to believe that athletes are more susceptible to infectious illness during intense exercise and competition, and there is a general (but still relatively unsubstantiated scientifically) belief that while physical fatigue may increase

susceptibility to infection regular exercise at moderate capacity may prevent common infections (Smith, 1995; MacKinnon, 1992).

Exercise effects natural immunity, T and B cell functions and cytokine responses through hemodynamic changes and by endocrine hormones secreted in response to physical stress. Research has characterized the general trends, but the complexity of the human immune system, makes deciphering the interaction of all of its subcomponents in response to exercise difficult (Hoffman-Goetsz & Pedersen, 1994). While the impact of exercise on the immune system may still be a clinical enigma, the intensity, duration and chronicity of the exercise seems to determine the magnitude of the effect of exercise on the immune system (Cannon, 1993; Hoffman-Goetsz & Pedersen, 1994). Intense exercise at maximal aerobic capacity has been shown to have the strongest impact, and suppressed several types of functional responses measured *in vitro* for up to several hours post exercise (Hoffman-Goetsz & Pedersen, 1994).

The biphasic nature of the response of many immune system components to exercise stress has lead to the suggestion of possible experimentally documented immunosuppression in vitro (Eichner, 1993). The response of lymphocytes to exercise provides an example of this response as there is a characteristic lymphocytosis during exercise followed by a lymphocytopenia during recovery (Nieman, 1997). It is the decrease of immune cell numbers and responsiveness during recovery that has lead to the proposal of the "open window hypothesis" to account for increases susceptibility to illness. This hypothesis suggests, the transient suppression of some immune parameters post exercise provides an open window where host protection is compromised and infection to take hold (Pedersen and Ullum, 1994).

Epidemiological data lends support to the proposed immunosuppression noted in vitro, and epidemiological evidence, both anecdotal and experimental, suggesting increased incidence of upper respiratory tract infections (URTI) in athletes has stimulated much of the increase in research into exercise and immunity in recent years (Nieman, 1997). Several studies have investigated possible mechanisms resulting in this increased susceptibility to infection, by studying the response of various immune components. The

results have not provided consistent evidence of immune suppression, but several patterns have emerged: Intense exhaustive exercise transiently alters a number of immune parameters such as circulating leukocyte subset numbers, cytokine concentrations, natural killer cell activities and neutrophil and macrophage activity and immunoglobulin secretions; some of the alterations in these parameters may remain through recovery for up to a day later;
some athletes exhibit low resting or post exercise levels of immune parameters suggesting immune function impairment; clinical studies suggest that this immune suppression may be relevant in affecting host protection (Nieman, 1997).

In reviewing the influence of exercise on the immune system it is important to discuss the effects observed in the literature on several components of the immune system to gain an understanding of the complete picture. The changes brought about by exercise stress varying in chronicity, intensity and duration will be discussed as they relate to changes in leukocyte subpopulation, lymphocyte proliferation, natural killer cell activity (NKCA), B cell function and neutrophil function. The potential mechanisms by which immunomodulation and increased susceptibility to infection is thought to occur will also be reviewed.

Leukocyte Subpopulations

Exercise is associated with an extensive change in white blood cell count, and while existing data on the response of some immune parameters seem to conflict, there exists a consistent leukocytosis in response to most types of acute exercise stress despite variations in intensity and duration of the exercise protocol (MacKinnon, 1992). This immediate leukocytosis consists primarily of increased lymphocyte counts as NK, T and B cells increase in the blood during exercise, while neutrophils account for the delayed leucocytosis as they increase during and following exercise. At the same time T cell relative composition changes (usually a relative increase in $CD8^+$ cells) leading to a decrease in the $CD4^+/CD8^+$ ratio. During recovery from severe exercise blood lymphocyte counts decrease below normal, with long duration high intensity exercise

showing the largest suppression (Hoffman-Goetz and Pedersen, 1994).

Long endurance exercise has been studied by a group of researchers who have investigated marathon runners extensively (Nieman, Berk, Simpson-Westerberg, Arabatzis, Youngberg, Tan, Lee & Eby, 1989; Nieman, Simandle, Henson, Warren, Suttles, Davis, Buckley, Ahle, Butterworth, Fagoaga & Nehlsen-Cannarella, 1995; Nieman, 1997). Following treadmill running for 2.5-3hrs to exhaustion the increase in total leukocytes (50-100%) peaked 3 hours post exercise and returned to normal by the next day. Within this increase leukocyte subsets respond differently, the granulocytes increased most (250%) along with monocytes (60%). At the same time the lymphocytes were observed to increase by 31% and during recovery lymphocytes exit the blood compartment decreasing by 19-40% from pre-exercise values (Nieman et al., 1989; Nieman, 1994). Of the three major lymphocyte subpopulations (T, B and NK), the NK cells are the most responsive to strenuous exercise and may increase 150-300% (Nieman, 1994). This post-exercise decrease in blood lymphocytes lasted for six hours and consisted of the T and NK cell subsets only. Neutrophils were observed to increase throughout exercise and recovery leading to a increase in the ratio of neutrophils to lymphocytes (Nieman et al., 1995). A competitive event may lead to even greater changes as the leukocytosis has been shown to increase in the order of 200-300% (Nieman, 1994).

This increase in leukocytes has been observed following other long duration tasks including a 250km bicycle race (404 min average duration) in 6 cyclists (Gannon, Rhind, Suzui, Shek and Shephard, 1997). This leukocytosis was followed by a lymphocytopenia consisting mainly of a reduction in T cells ($CD3^+$) and the $CD4^+$ T cell subset. The most pronounced depression of lymphocytes following endurance exercise was documented by Shek, Sabiston, Buguet and Radomski (1995). In a study on treadmill running for 2 hours at 65% of VO_2 max, the observed leukocytosis (mainly granulocytes and lymphocytes) was followed by a significant decrease in the concentrations of T and NK cells. During the exercise an increase was observed in $CD3^+$, $CD4^+$, $CD8^+$ and $CD16^+$ (NK) cells (NK showed the greatest increase), following exercise a progressive decrease in T cells was

observed as the counts were less than 60% of pre exercise value by 2 hours post. The NK cells demonstrated a persistent decrease following exercise and a decrease of 40% was observed for as much as 7 days following. This finding has been suggested to be a spurious one as most research has shown a return to baseline values by the following day. These general trends of immediate and delayed leukocytosis, delayed neutrophilia, and delayed lymphocytopenia have been demonstrated by others with various exercise protocols. Kettler, Gabriel, Brechtel and Kindermann (1996) studied exhaustive exercise at anaerobic threshold (AT) in triathletes; Bury, Louis, Radermecker and Pirnay (1996) studied running over the range of 45-75% VO_2 max (with suppression only following the most intense exercise); and Bioux, El Mezouini and Orsetti (1995) investigated 2.5 hours of swimming to exhaustion in rats.

Moderate intensity exercise in general has been shown to induce smaller changes in immune cell subpopulations than strenuous exercise. Gabriel and Kindermann (1997) distinguished between moderate exercise which has a duration of < 2 hours at 85% of AT or <30 min at AT, and strenuous exercise which was defined as exhaustive exercise at AT or above, and exhaustive long duration exercise of 2-3 hours. Moderately intense exercise resulted in a smaller leukocytosis, lymphocytosis and neutrophilia, and lymphocytopenia during recovery, and this was suggested to be dependent on exercise induced changes in the stress hormones epinephrine and cortisol. Changes in the concentration of neutrophils seems to depend on duration rather than intensity, as the rise in neutrophils and monocytes is greater following long duration exercise.

The responses of lymphocyte subsets have also been observed in shorter duration exercise tasks. Resistance exercise was studied in subjects performing leg squat exercise at 65% of 1 RM (sets of 10 with 3 minutes rest to failure). A leukocytosis was observed during exercise including a lymphocytosis, followed by a lymphocytopenia during recovery (Nieman, Henson, Sampson, Herring, Suttles, Conley, Stone, Butterworth and Davis, 1995). Intense anaerobic interval training consisting of 15 x 1 minute running intervals at 95% of VO_2max has been shown to induces changes in the composition of total lymphocytes (Hinton, Rowbottom, Keast and Morton, 1997). Significant increases

in NK ($CD56^+$) and T suppressor ($CD8^+$) cells increased immediately post exercise and T helper ($CD4^+$) cells decreased as did the $CD4^+/CD8^+$ ratio. A study that provides a more detailed picture of the immune response to shorter duration exercise investigated the response to bicycle exercise at 80% of VO_2max to exhaustion in 12 cyclists, repeated by 6 of the subjects following one hour of recovery (Field, Gougeon and Marliss, 1991). Significant increases ($p<0.05$) in the following cell population were illustrated at the end of exercise: total leukocytes (2x), monocytes (1.9x), neutrophils (1.9x) and lymphocytes (2.3x). The changes had returned to baseline values by one hour of recovery with the exception of lymphocytes which decreased below the pre exercise level. Following the second exercise bout the leukocytes and neutrophils remained elevated after the one hour recovery period, and lymphocytes increased less than during the first, and again decreased below the pre exercise value during recovery. When the lymphocyte subsets were investigated, NK ($CD56^+$) and T suppressor ($CD8^+$) cells increased , and the % of T cells ($CD3^+$), $CD4^+$ T cells and $CD4^+/CD8^+$ ratio decreased at exhaustion.

Whether these changes in immune cell concentrations and relative distributions compromise host protection is not clear. But, the consistent trend in $CD4^+/CD8^+$ ratio to decrease has been associated with depressed immune function in diseased states, and Nieman (1994) stated that "having less T cells than normal for several hours of recovery is the same as having less soldiers on the front lines of a battlefield".

The responses of leukocyte subsets to training have been studied longitudinally as a means of investigating potential benefits of exercise in improving baseline immune function. The results of the research performed thus far has demonstrated some conflicting responses, but the generally, improvement in immune function has not been documented following training of 4-18 weeks duration. 8 weeks of training in 16 breast cancer patients did not induce changes in NK or T cell concentrations in peripheral blood (Nieman, Cook, Hanson, Suttles, Rejeski, Ribisl, Fagoaga & Nehlsen-Cannarella, 1995). Lotzerich (1996) was unable to observe changes in leukocyte subsets throughout a track season in female short distance runners. 12 weeks of moderate training did not alter total lymphocyte counts in 11 male subjects (Mitchell, Paquet, Pizza, Starling, Holtz and

Grandjean, 1996). In contrast, a 10 week aerobic training program was shown to increase resting $CD2^+$, $CD4^+$, $CD45RA^+$ $CD4^+$, $CD8^+$ and $CD20^+$ counts in 14 inactive males (LaPierre, Antoni, Ironson, Perry, McCabe, Klimas, Helder, Schneiderman and Fletcher, 1994). While a training program failed to improve immune parameters in previously sedentary elderly women, the baseline immune function in physically active elderly women was shown to be superior which suggests some benefit of long term activity not detected in the shorter duration training period (Nieman, Henson, Gusewitch, Warren, Dotson, Butterworth and Nehlsen-Cannarella, 1993).

Lymphocyte proliferation

The determination of the proliferative response of human lymphocytes upon stimulation with various mitogens in vitro is well established as a test to evaluate the functional capacity of T and B lymphocytes (Nieman, 1997). When lymphocytes encounter a foreign pathogen their capability to secrete cytokines and divide is an essential part of the adaptive immune system, and the stimulation of lymphocytes with mitogens is believed to simulate the events following antigen stimulation in vivo. Following proliferation (one, two or three days) of the lymphocytes in response to stimulation with a mitogen, the cells are pulsed with methyl-^{3}H thymidine and incubated further. The incorporation of this radioactive label into the DNA of the cells is counted in a liquid scintillation counter (Nieman, 1997). The response of the lymphocyte population in proliferative assays depends on the mitogen which the researchers used as that effects which subpopulation of cells is stimulated (Hoffman-Goetz and Pedersen, 1994). Concanavalin A (ConA) stimulated assays show decreased proliferation following exercise suggesting impact on the T cell, as do phytohemaglutinin (PHA) stimulated assays due to the $CD4^+$ specific T cells. Pokeweed mitogen (PWM) is generally thought of as a B cell mitogen, but also stimulates T cells, and phorbol myristate acetate with ionomycin (PMA + iono) stimulate most cell types. Proliferation assays using these mitogens also tend to decrease following exercise. In contrast, IL-2 or lipopolysaccharide (LPS) stimulation show an increase in proliferative response

following exercise. This has been attributed to an increase in the proportion of NK ($CD16^+$) cells in the assay, as these cells are responsive to LPS and IL-2 (Field et al., 1991; Hoffman-Goetz and Pedersen, 1994).

Intense exhaustive endurance exercise has the largest negative impact on the proliferation of lymphocytes. In a study investigating the impact of 2.5 hours of running on lymphocyte proliferation a decrease in Con A stimulated lymphocyte proliferation of 30-40% was observed for more than three hours post exercise (Nieman et al., 1995). This decrease in function is much more prolonged than that described after exercise of less than one hour duration, and is related to numerical shifts in immune cells (decrease in $CD3^+$ T cells) (Nieman et al., 1995; Nieman,1994).

Shorter duration intense exercise have also been demonstrated to influence the proliferative response of lymphocytes to stimulation post exercise. Hinton et al. (1997) showed that PHA stimulated blastogenesis decreased for a period of 30 minutes following an intense interval workout. This decreased activity is likely due to changes in the proportion of activated cells in the assay (large increase in NK), as isolated purified lymphocytes ($CD4^+$ and $CD8^+$) showed no significant changes during exercise. Resistance exercise lead to changes in ConA stimulated lymphocyte proliferation, but again this difference was not apparent when expressed on a per T cell basis (Nieman et al., 1995) . Field et al. (1991) investigated the proliferative response of lymphocytes to several mitogens (Con A, PMA, PHA, PWM) in response to a maximal bike test at 80% of VO2max (12.9 min average duration). The lymphocyte response to all mitogens decreased ($p<0.05$) immediately post exercise and recovered to pre exercise values by one hour of recovery.

Whether these studies show immunosuppression is unclear as the changes in lymphocyte proliferation are related to changes in relative proportions of cells in the assay, namely the large NK cell increase leads to a lower proportion of T cells in the assay and this must be considered when interpreting results (Pedersen Rhode and Zacho, 1996).

Natural killer cell activity

Specific effects have been shown on the population of cells known as natural killer (NK) cells. The absolute concentrations and relative fraction of peripheral blood mononuclear cells with NK markers increase during exercise. NK cells are large granular lymphocytes that can mediate cytolytic reactions against a variety of neoplastic and virally infected cells (Nieman, 1997). They also show non-cytolytic function by inhibiting microbial colonization and growth of bacteria, fungi, viruses and parasites. NK cells represent 10-15% of blood lymphocytes in the resting state.

Functional assays have also shown a spontaneous increase in the natural killer cell activity (NKCA) immediately after exercise, likely linked to %NK cells in the assay (Hoffman-Goetz and Pedersen, 1993). The increase in activity is followed by a decrease in activity below baseline values during recovery due to numerical shifts which decrease the number of NK cells in the assay. Intense 2.5 hour running has produced an increase in NKCA immediately after exercise followed by a 40-60% decrease lasting up to 6 hours in recovery (Nieman et al., 1995; Nieman, 1997). Shek et al. (1995) documented a decrease in NKCA lasting up to a week following exhaustive endurance exercise lasting 2 hours. Exercise at an intensity of 80%VO2max has been reported to lead to an increase in NKCA (Hoffman-Goetz & Pedersen, 1993). This is supported by Field et al. (1991) who demonstrated that during bike exercise lasting an average of 12.9 minutes NKCA was increased immediately following exercise and returned to baseline within one hour of recovery.

In Nieman's studies on marathon runners one interesting finding is that the baseline NKCA of marathon runners were greater than that measured in controls suggesting some advantage conferred by fitness and training.

B cell Function

A major function of the immune system is the production of soluble components; of which immunoglobulins with antibody activity are of primary importance. Antibodies are produced by plasma cells (end stage B lymphocytes) in response to a foreign

substance (Nieman, 1997). The impact of exercise on the B cell population has not been well studied although the general trend is for the number of immune cells to increase with exercise (Hoffman-Goetz and Pedersen, 1993). Nieman (1997) stated that overall, the literature to this point suggest no significant impairment of antibody production after vaccination following endurance exercise. However, the salivary IgA concentration was shown to decrease after a race in cross country skiers, with no change in the proportion of CD 19^{+} (Tomasi, Trudeau, Czerwinski & Erredge, 1982).

Neutrophil Function

Neutrophils make up 55-65% of blood leukocytes and monocytes compose another 3-9%. These phagocytic cells have an important role in non specific or innate immunity. Phagocytes form the first line of defence to infectious agents and are also involved in muscle tissue inflammatory response to exercise induced injury (Smith, 1997; Suzuki et al., 1996). The function of phagocytic cells such as neutrophils consist of several stages: adherence, chemotaxis, attachment, ingestion and killing of the foreign antigen (Rincon, 1994). The majority of studies assess function of these cells using tests to determine the ability to engulf pathogens (phagocytosis) or facility to kill pathogens once engulfed (oxidative burst) (Nieman, 1997). Compared to other immune parameters relatively few studies have examined the effect of prolonged intense exercise on phagocytic function (Pyne, 1994; Pyne, Baker, Fricker, McDonald & Nelson, 1994; Rincon, 1994).

In general it has been observed that both moderate and high intensity exercise performance is associated with an increase in the blood granulocyte and monocyte phagocytosis and degranulation. Moderate exercise (less than 60% MAP) tends to enhance oxidative burst, while during high intensity exercise it is decreased (Rincon, 1994; Nieman, 1997; Smith, 1997). It can be seen than in terms of neutrophil function it is not uncommon to see two indices of function respond to exercise in opposite directions (Smith, 1997). In response to 2.5 hours of intensive running granulocyte phagocytosis are increased 45%, while oxidative burst activity decrease 14% by 6 hours post exercise.

Bury and Pirnay (1995) studied the immune response of neutrophils to endurance exercise by investigating the plasma myeloperoxidase (MPO) concentration as a marker of degranulation. Subjects performed exercise trials of 4, 3, and 2 hours at 45, 60 and 75% of VO_2 max respectively. The concentration of MPO increased following each exercise trial, but was not correlated with an increase in neutrophil count suggesting that degranulation is independent of mobilization following this long duration endurance exercise. Suzuki, Naganuma, Totsuka, Suzuki, Mochizuki, Shiraishi, Nakaji & Sugawwa (1996) suggested that at a relatively higher intensity of exercise (70% VO_2 max) of 1.5 hours duration repeated over 7 consecutive sessions, the increase in reactive oxygen species by neutrophils is related to the large increase in neutrophil numbers mobilized in response to exercise.

The potential immunosuppressive effects of exhaustive endurance exercise on oxidative burst of neutrophils measured by flow cytometry is demonstrated by a study in which triathletes exercised at AT until exhaustion. A significant increase in neutrophils was observed, but this increase was accompanied by a reduced generation of super oxide anions suggesting a reversible immunosuppression (Kettler et al., 1996). During shorter duration exercise some neutrophil functions have been shown to increase. Following a 14 minute maximal bicycle ergometer test the generation of reactive oxygen species increased 52% (Huupponen, Makinen, Hyvonen , Sen, Rankinen, Vaisanen and Rauramaa, 1994). A different response was observed in a study looking at the effects of intense graded exercise treadmill running to exhaustion in which neutrophils microbicidal activity was decreased immediately post exercise, and elevated 24 hours later (Hack, Strobel, Rau and Weicker, 1992).

It has been suggested that chronically, periods of strenuous training in athletes may suppress neutrophil activity . Studies on both male basketball players and female track athletes have shown mixed results on the function of neutrophil cells. During the basketball season, measures of bactericidal activity and superoxide release increased, while the cellular adhesion decreased during the championships (Benoni, Bellavite, Adami, Chirumbolo, Lippi, Broddo, Giulini,& Cuzzolin,1995). A period of training lead

to a decreased phagocytic activity of granulocytes in female short distance runners (Lotzerich, Wilczkowiak, Stein, Peters, 1997). Overall, the response of exercise on neutrophil function is dependent on duration and intensity , and the conflicting responses on different functional measurements make the overall impact difficult to discern. Rincon (1994) suggests that the stimulation of certain stages of the phagocytic process may counterbalance the documented decrease in lymphocyte activity brought about by exercise.

Proposed mechanisms of susceptibility to infection in athletes

Several mechanisms have been proposed to explain the response of the immune system to exercise stress in the sense that on one hand exercise is thought to be beneficial to infection resistance, and on the other, strenuous exercise may depress certain immune functions, increasing the risk of infection.

A 'J-shaped' model has been proposed by Nieman and Cannarella (1994) to explain the relationship between regular exercise and risk of infection such as URTI, it has also been referred to as 'U-shaped' by Hoffman-Goetz and Pedersen (1993). The risk of infection is given as average for more sedentary individuals and decreases in those who engage in moderate intensity exercise, while the risk is highest for those who participate in high intensity exercise leading to exhaustive fatigue.

The 'Open window' hypothesis proposed by Pedersen and Ullum (1994) was proposed to explain how the experimentally observed responses of immune parameters *in vitro* could lead to the increased risk of infection suggested above. During moderate exercise responses such as NKCA increase during exercise and decrease to resting levels after, while during more severe exercise, responses such as NKCA increase during and decrease below pre exercise values for a time period during recovery. This period of relative immunosuppression is thought to 'open the window' for infection to take hold.

The role of hormonal interaction with and regulation of the immune system can be illustrated by the observation that cortisol increases with increasing exercise intensity, and that this hormone has immunosuppressive activity (Weicker & Werle, 1991). Cortisol

and epinephrine are the primary hormones suggested to have a role in modulation of the immune system, as cortisol and epinephrine increase during exercise at an intensity >60% of VO2max. The increase in cortisol has been correlated with changes in leukocyte subsets such as the increase in neutrophils and decrease in lymphocytes following 2.5 hours intense running (Nieman et al., 1995). Pedersen, Bruunsgard, Klokker, Kappel, Maclean, Nielsen, Ullum and Zacho (1997) summarized the effect of neuroendocrine factors on exercise induced immune changes. They concluded that the exercise-induced changes in leukocyte subsets and function involves a mulitfactorial mechanism involving hormonal, metabolic and physiological changes with exercise. Adrenaline and to a lesser extent noradrenaline are involved in modulating changes to lymphocyte subsets and NKCA, while the increase in those catecholamines and cortisol are involved in the neutrocytosis. Cortisol is the hormone primarily involved in lymphocytopenia.

The proposed mechanistic models of the impact of exercise stress on the immune system leading to decreased resistance to infection are discussed above. In addition, specific events have been suggested which may combine to make the athlete susceptible to URTI after a competitive event. They are as follows: the high ventilatory flow rates alter the mucosal surfaces of airways and thus the first line of immune protection; the suppression of the immune system due to chronic exercise stress by any or all of the above proposed mechanisms; the depletion of important factors for proper immune function and in addition the additive effect of psychological stress (MacKinnon, 1997).

It can be seen that the nature of the immune response is sensitive to the type, duration and intensity of exercise being performed. The need to develop a consistent protocol and distinguish between effects seen immediately following the exercise and the day following recovery is clear if a more complete understanding of the relationship is to be gained. The suggestion that the response of the immune system to exercise stress is similar to other stressors studied as subdisciplines of stress immunology, illustrates the potential for additive effects of different stressors (Hoffman-Goetz & Pedersen, 1993).

Perna, Schneiderman and LaPierre (1997), also suggested the similarities between exercise and other stressors and the potential additive effects leading to a more pronounced immunosuppression. In suggesting future directions of research Hoffman-Goetz and Pedersen (1994) defined four different types of exercise situations which would place substantially different stresses on the immune systems. The first involves acute time limited exercise such as the weekend exerciser; the second, chronic intermittent exercise stress (regular episodic exercise); the third, chronic exercise stress such as the elite athlete is subjected to seasonally; and the last chronic exercise stress with superimposed acute episodes such as competition. Most research has concentrated on the first and third exercise situation. The development of a model of exercise that includes the physical and psychological factors (closer to the competitive situation) that influence exercise activity and immune functions is needed to get a better understanding of the immune response in the competitive athlete (Hoffman-Goetz & Pedersen, 1993). The design of our study aims to achieve this through the combination of stresses facing the lightweight rower (exercise stress, and both the physical and psychological stress of 'making weight').

2.4 Gender Differences in the Immune Response

The increase in research on the impact of exercise stress on the immune system over recent years, has for the most part been performed on male subjects. Research investigating the immune response to exercise in female subjects is very limited. Highly conditioned active elderly women have been shown to have superior immune function to sedentary women of similar age, and a 12 week training intervention did not result in an improvement in immune function (Nieman et al., 1993). The effect of moderate exercise training over eight weeks on NKCA was studied in breast cancer patients, and no improvements were observed (Nieman et al., 1995). Young female gymnasts studied by Eliakim, Wolach, Kodesh, Gavrieli, Radnay, Ben-Tovim, Yarom and Falk (1997), and their immune responses showed no difference from untrained controls. These studies represent the small body of research on exercise immunology in female subjects, which is

in stark contrast to the many studies performed on male subjects.

The differences in sex hormones between men and women are obvious, and the influence of sex hormones on the immune system has been studied. Research has also shown differences in the subpopulations of immune cells and functional differences in the activities of these cells between the sexes. Lymphocyte numbers have been shown to be lower in the female, NKCA may also be lower, and the cell mediated response may be decreased (Giglio et al., 1994). These observations combined with the evidence for an increased risk of autoimmune disorders in women have lead to the suggestion that an "immunological dimorphism" exists between men and women (Chao, 1996; Giglio et al., 1994). The involvement of sex hormones in the observed differences in immune function between the sexes has been suggested based on evidence that estradiol inhibits $CD8^+$ lymphocytes and NKCA; and, the presence of estrogen receptors on $CD8^+$ lymphocytes and prolactin (PRL) binding sites on B and T lymphocytes (Giglio et al., 1994). While the communication between endocrine and immune systems is not disputed, the extent and significance of immune system changes throughout the menstrual cycle are not clear. In addition, whether these changes may result in changes in the response to exercise is not well known.

Giglio et al. (1994) investigated the relationship between hormone levels and immune functions in several different groups of women and men ranging from young to old, pre and post menopausal, and those with premature menopause. They were trying to understand why women were more prone to the onset of autoimmune disease than men, a difference which disappears after menopause. In doing so, these researchers showed that the total number of lymphocytes decreased in older post-menopausal females and that this immune parameter was higher in men than women. They also showed a subpopulation of T cells ($CD2^+$) decreased in the older groups and in premature menopause and again had lower levels of this cell type than men. The natural killer cell activity of men was observed to be lower in females than in males and was relatively constant among the group (as was number and %) but it increased in those with premature menopause. The number of total, $CD2^+$, $CD4^+$ T lymphocytes, B and NK cells was directly correlated with

LH and negatively with FSH. $CD2^+$, $CD4^+$ and B lymphocytes correlated positively with PRL, while $CD8^+$ and B lymphocytes were inversely correlated with FSH and $17BE_2$ levels. This data suggest that sex hormones have the ability to influence changes in immune cells, and the lowering the risk of autoimmune disorders during and after menopause is thought to be due to increased FSH and decreased in PRL leading to a decrease in B and $CD4^+$ T lymphocytes .

Chryssikopolous (1997) reviewed the two way communication between the immune and endocrine systems. He concluded that not only does the immune system influence the endocrine system, but the endocrine system can also influence the immune system in return. This is illustrated by the influence of cytokines on the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-ovarian axis, and the ability of ovarian and adrenal hormones to influence secretions of cytokines by cells of the immune system.

The potential for communication between the immune system and the hypothalamic-pituitary-ovarian axis, provides some rationale that the immune system's functional response to exercise could change throughout the menstrual cycle. Recently, a study was published that investigated the effects of a relatively high intensity exercise bout on leukocyte and lymphocyte subpopulations and the circadian variations involved in female subjects (Zelazowska, Singh, Raybourne, Sternberg, Gold & Deuster, 1997). These researchers acknowledged that not controlling for the phase of the menstrual cycle of the subjects was a limitation of their study, but measured the estrogen and progesterone concentrations to control for this limitation. Absolute counts for leukocytes and lymphocyte subpopulations increased following exercise (with the exception of monocytes in the AM). The lymphocyte subpopulations decreased below baseline values after a 40 minute recovery period. Circadian variations were noted in absolute counts for total T cells, T helper cells and B cells. The counts for these cells were significantly higher in the PM, but a key finding of the study is that the magnitude of the response of the immune system to exercise did not change despite baseline circadian variation. Only NK cells exhibited a circadian variation in exercise response as the PM sample had

higher baseline relative counts and response to exercise than the AM. The concentrations of progesterone and estrogen measured in this study did not correlate with any of the lymphocyte subpopulations studied, suggesting that limited changes in immune parameters throughout the menstrual cycle.

In support of this observation that the immune system may not exhibit major changes throughout the menstrual cycle is research published by Northern, Rutter and Peterson (1994). They investigated the counts of leukocyte and lymphocyte subsets during 24 hour (daily) and 28 day (menstrual) cycles in 5 subjects. They demonstrated that for the most part baseline and circadian patterns in levels of total lymphocyte and lymphocyte subsets are not affected by the menstrual cycle. The total leukocyte count was not different from day 6 compared to day 22 (6.15±1.96 vs 6.39±2.14 x 10^{-3} cells/μl), as were group means for granulocytes, monocytes, lymphocytes, T cells, B cells, T helper and suppressor subsets and NK cells. In addition, they showed that the variation in lymphocyte counts caused by menstrual cycle was much smaller than differences between individuals on the same day of the menstrual cycle (leukocyte counts ranged from 3.63 to 8.60 at day 6 and 3.75 to 9.45 x 10^{-3} cells/μl on day 22). However, some significant differences were found in circadian variations in several cell types, and significant differences were detected between the day 6 and day 22 time point in the circadian pattern of total leukocyte, granulocyte, monocyte and NK cells. The difference in monocytes was detected at noon with higher counts on day 22, as was the difference in counts for total leukocytes and granulocytes, the difference in NK cells was at the 18 hour time point again higher for day 22. Significant differences were not detected at the other time points. Other researchers have suggested that circadian changes in immune function throughout the menstrual cycle such as a decrease in NKCA do occur and have been attributed to increased progesterone and decreased stage IV sleep (Moldofsky, 1994).

The differences in the immune systems between the genders has been well documented and changes in the status of the females immune system throughout the menstrual cycle may occur. Whether any possible changes in the immune system throughout the menstrual cycle will change the response of the immune system to

exercise stress is not well understood, and only limited data at this point exist suggesting that it does not. In light of this information more research is needed into the impact of exercise stress on the female athlete. Furthermore , the response of the immune system to an intense aerobic power rowing performance in female subjects has not been studied.

2.5 Effects of Weight Loss on the Immune System

It is well recognized that proper nutrition is essential for a well functioning immune system. Immune function of hospitalized patients and citizens of third world countries have been shown depressed, and this has been attributed to protein malnutrition (Chandra, 1983). Dietary problems need not reach the level of extreme malnutrition in order to alter immune function, as simple caloric restriction has been shown to alter immune function (Field, 1991). Research on the impact of weight reduction on the immune system has focussed on caloric restriction through dieting in obese subjects. The impact of acute rapid weight reduction, such as that practised by lightweight rowers through food and fluid restriction has not been studied in the literature. This section of the review of literature will address research investigating the effects on the immune system of weight reduction via prolonged diet in obese subjects, caloric restriction in military trainees, short term dehydration and short term weight reduction via calorie restriction in athletes.

Kelley et al., (1994) investigated the immune response in obese women over a prolonged (12 week diet) with moderate (50%) energy restriction. They assessed cellular immunity via immunophenotyping of lymphocyte subsets and lymphocyte blastogenesis in response to mitogen and humoral immunity through assays for immunoglobulins and complement. There was no difference whether the subjects received a high fat or low fat diet, but overall an energy restrictive diet lead to decreased serum concentration of IgG, IgA, C3 and the number of circulating NK cells. No change was observed in lymphocyte subsets or proliferative responses over the course of the calorie reduction period. In a similar study, the calorie restriction was performed over a shorter period (6 weeks) with a very low energy diet (but with adequate nutrient and protein) on 11 females and 1 male

(Field, Gougeon and Marliss, 1991). A significant decrease in total leukocytes, neutrophils, lymphocytes and monocytes was observed from week one of the diet and onward. There was no change in lymphocyte subsets with the diet except for a small decrease in $CD4^+$ T cells, causing the $CD4^+/CD8^+$ ratio to decrease. In addition, proliferation of peripheral blood mononuclear cells in response to several mitogens decreased after week one (PMA +iono) and week six (Con A, PHA and PWM). Similar results were also found in another study in which 13 obese females dieted over 12 weeks using caloric restriction (Nehlsen-Cannarella, Nieman, Hanson, Butterworth, Fagoaga, Warren and Rainwater, 1997). A 9.4kg weight loss was associated with significant decreases in T, B, monocyte and granulocyte function (oxidative burst and phagocytosis).

The extent to which calories are restricted was shown to be related to the amount of immunosuppression observed in a study on military trainees (Kramer, Moore, Shippee, Friedl, Martinez-Lopez, Chan and Askew, 1997). In this study, two groups of military trainees (n=50, n=55) were put through a combination of stresses including caloric restriction and sleep deprivation. The proliferation of lymphocytes in response to PHA and TT were decreased with diet, and the second group showed a lesser degree of immunosuppression related to a smaller caloric restriction.

The effect that dehydration may have on the immune system has not been clearly investigated in the literature, but Greenleaf et al. (1995) provided evidence that the stress of dehydration may impact the immune system. The researchers examined the effect of rehydration before exercise in four dehydrated men on the $CD4^+/CD8^+$ lymphocyte ratio. The rationale for the study concerned the fact that astronauts have been shown to be both dehydrated and immunosuppressed following spaceflight (Greenleaf, 1990; Cogoli, 1981), and that the stress of dehydration may be part of the mechanism involved in changes in the immune system. In this study, 24 hours of voluntary fluid restriction prior to exercise resulted in a decreased $CD4^+/CD8^+$ ratio. The ratio observed pre-exercise was 0.77 vs normal values of 1.2-1.5. Although the purpose of the experiment was to investigate the effect of rehydration immediately prior to supine submaximal exercise in dehydrated men, they performed a regression analysis of the $CD4^+/CD8^+$ ratio on mean

plasma osmolality and found an regression coefficient of -0.76 suggesting a relationship between increased immunomodulation with increased dehydration. They concluded that the level of subject hydration and mean plasma osmolality may be factors involved in the mechanism of immune system modulation by exercise.

The only study using athletic females as subjects when investigating the effects of weight reduction on the immune system looked at a two week hypocaloric diet during training in nine athletes from various sports (4 gymnasts, 2 swimmers, 2 volleyball players and 1 tennis player) (Kono et al., 1988). These researchers used a two week period of controlled diet where the subjects received 2000 kcal/day while they trained, followed by a two week period where the diet was cut back to 1300 kcal/day. On average, this two week diet lead to a 2 kg decrease in weight of the subjects. The immune status of the athletic females, as indicated by function of monocytes, decreased over the 2 week hypocaloric diet. The phagocytic function of monocytes against zymosan particles was higher than controls before the diet, but it decreased significantly during the diet. The phagocytic function against sheep erythrocytes was not different than controls pre diet, but it also decreased over the diet period. The lymphocyte proliferation against PHA also decreased with the calorie restriction.

Together the previous studies show that potential of calorie restriction and hypohydration to impact the immune system in both obese and athletic subjects. While this research is longer term than the proposed study on rowers and primarily involved caloric restriction as a means of weight loss, it demonstrates the importance of proper nutrition and hydration to the health of the immune system.

2.6 Clinical and Epidemiological Significance of Immunosuppression

Epidemiological evidence has suggested a positive role for exercise training in the prevention and treatment of certain illnesses. Thus far, research has suggested an association between regular exercise and reduce incidence of cancers, and overall animal studies have shown that exercise training has conferred some resistance to tumor growth (MacKinnon, 1992). In addition, exercise is being used in the treatment of diseases such

as cancer and AIDS (MacKinnon, 1992; Nieman et al., 1995; Lawless, Jackson & Greenleaf, 1995). Although exercise may be beneficial in some respects to treat or prevent illness, the focus of this review will concern evidence that shows impairment of host protection in the athlete.

Data from clinical and epidemiological studies can help to clarify findings from experiments performed *in vitro* on peripheral blood samples by providing evidence of the immunosuppression that is assumed or predicted by changes in immune parameters. It is reasonable to suggest that if host protection is shown to be diminished, some aspect of altered immunity would at least be partially responsible (Nieman, 1997). Thus far, experimental evidence is not clear as differences in study methodology make comparisons between studies difficult (Smith, 1995). Investigations have looked for increased incidence of illness in a specific group of athletes that would suggest impaired immune function, or show the significance of an experimentally observed alteration in immune response. There is a general belief that the incidence of illness is increased in elite athletes, especially during periods of intense training and competition (MacKinnon, 1992), and URTI seems to be the primary illness reported by athletes (Nieman, 1997; Peters, 1997). While much of the evidence suggesting athletes are more susceptible to illness is anecdotal, there are two key studies that demonstrated a significant increase in incidence of URTI in ultra marathon and marathon runners (Peters and Bateman, 1983; Nieman, Johanssen, Lee and Cermak, 1990) .

Ultra marathon runners (n=140) were studied before and after a 56km race with questionnaires concerning symptoms of URTI (Peters and Bateman, 1983). Each runner had an age matched nonrunner living in the same house as a control. During the two weeks following the race the runners experienced an increased incidence of URTI compared to the controls (33% and 15% respectively). The faster athletes seemed to have a greater incidence of illness as URTI symptoms correlated with race time ($r=0.995$, $p<0.01$). These results are similar to those found by Nieman et al. (1990) as they investigated an even larger group of marathoners (n=2311) in preparation for the 1987 Los Angeles Marathon. One week following the race, 12.9% of runners reported illness

compared to 2.2% of control runners who did not participate. The runners who had trained >96km/week doubled their chances of illness compared to those who trained <32km/week.

These studies together suggest an increased risk of illness in elite endurance athletes that is related to training volume, competition stress and racing intensity. This increased risk of illness has not been demonstrated in other less intense, or shorter duration exercise activities (MacKinnon, 1992).

2.7 Consequences of Weight Reduction via Hypohydration

The effect that a reduced body water content may have on the performance of an exercise task has been examined in detail in the literature from several different perspectives. Hypohydration can effect several of the body's physiological systems. It can also effect an athlete's psychological state and manifests these effects by directly affecting the performance of exercise tasks. Research has also examined different methods of hypohydration and the specific effects of each. Since 1947, when Adolf and associates published " Physiology of Man in the Desert", much research has been performed concerning the possible consequences that the loss of body water through sweat, or fluid restriction can have on performance of various exercise tasks and on the physiological functioning of the body's cardiovascular, musculoskeletal and thermoregulatory systems. The research which has the most direct application to the situation of the lightweight rower is that performed on wrestlers and the consequences of making weight. The weight loss is often achieved via a combination of food and fluid restriction, sweating and exercise, but for the purpose of this review, emphasis will be placed on the influence of hypohydration rather than caloric restriction to make weight.

Physiological Effects of Hypohydration

Body fluid cannot be lost and not replaced while maintaining both plasma volume and cellular hydration (Tipton, 1982). It is this change in body fluid homeostasis, typically brought about by fluid restriction and sweating, that triggers the characteristic physiological responses of hypohydration that are a concern for athletes. Hypohydration is thought to result in an increased heart rate at rest and during submaximal exercise, possibly due to a reduction in stroke volume; decreased blood pressure, plasma volume, muscle water content, blood flow to skin, blood flow to muscle; and as the dehydration becomes more severe, a decrease in sweat rate, muscle mineral content and blood flow to the kidneys can occur (Tipton, 1982; Sawka, 1992). These changes have an impact on aerobic performance and may lead to an increased chance of hyerthermia. The extent to which hypohydration will impact the individuals physiological responses and any exercise performance they undertake is dependent on the environment, with warmer temperatures increasing the risk, as thermoregulation and exercise performance in the heat are critically linked to body hydration status (Sawka, 1992).

The extent to which plasma volume is reduced depends on the amount of weight lost. In the only published study investigating hypohydration in rowers a body weight decrease of 5.16% (over 24 hours) by food and fluid restriction with light exercise lead to a plasma volume reduction of 12.5% (Burge et al., 1993). In another study cyclists were dehydrated by diuretics resulting in a 3% decrease in body weight that lead to a 15.3% decrease in plasma volume (Claremont, Costill, Fink & Van Handel, 1976). In a study where thermal dehydration was used, a body weight reduction of 4% lead to a plasma volume decrease by 12% (Costill & Sparks, 1973). It seems clear that a reduction in plasma volume occurs regardless of the method of dehydration, but how the weight is lost may effect the partitioning of body water loss from each of the major body fluid compartments.

It has been suggested that 93.6% of the weight lost during rapid weight loss protocols is from body fluid, the extent to which plasma volume, extracellular (interstitial fluid) or cellular water is reduced is a source of some debate (Costill, Cote and Fink, 1976). The proportion of total body water which was lost from the plasma during

hypohydration in rowers was estimated to be 12.1% (Burge et al., 1993). This result was very similar to the 12% value reported by Costill, Cote and Fink (1976). As most of the water lost during a combined exercise and fluid restriction protocol is from sweat, which is hypotonic to plasma, the result is a hypertonic hypovolemia (Kirby &Convertino, 1986). It is this situation which allows the protection of plasma volume at the expense of interstitial and intracellular fluid. When body water losses are not large the majority of water lost comes from the extracellular compartment rather than the intracellular fluid (60%-30%), but as the losses are greater, the distribution of lost fluid reverses (50%-40%), but plasma volume remains a small contribution (~10%) to the water loss (Costill & Fink, 1976; Sawka, 1992). Diuretic-induced hypohydration clearly effects the body fluid in a different manner as an isotonic hypovolemia is the result leading to a greater proportion of the fluid loss from the plasma (Sawka, 1992). The impact that the body fluid losses have on each of the body's fluid compartments is a source of some disagreement in the literature. The discrepancies in observed plasma volume changes may be dependent on the method of weight loss and if exercise is used, the intensity and duration of exercise used to induce the weight reduction (Kozlowski & Saltin, 1964).

Despite the relative protection of plasma volume at the expensive of intracellular and extracellular fluid, it is the reduction of plasma volume with dehydration that leads to the negative impact on exercise performance. Heat stress can lead to competition for a reduced blood volume between the muscles and the cutaneous vascular beds. This competition can impair heat loss through both dry and evaporative heat exchange; and, decrease central venous pressure, venous return and thus cardiac output (Sawka, 1992). Adolph and associates (1947), in their initial studies in "Physiology of Man in the Desert" were the first to attempt to determine a critical threshold for hypohydration to impact the cardiovascular system. They suggested that fluid loss of less than 2% of initial body weight had minimal effects on cardiovascular function as with this level of hypohydration there is little change in plasma volume. Beyond this level changes can be observed in the submaximal exercise response of decreased stroke volume, increased heart rate and decreased cardiac output. Allen, Smith and Miller (1997), showed that at a body weight

decrease of 3-5% that the heart rate increased related to a decreased stroke volume and that only at a 6-7% decrease in body weight would the changes in cardiac output be observed.

Body fluid losses do not only impact cardiovascular performance. A decrease in the volume of circulating blood may impair the conductance of heat from active tissues to the skin and respiratory systems to be dissipated. In addition a decrease in heat loss via sweating may also occur when plasma volume decreases, and this leads to an increase in core temperature. The mechanism of the decreased sweating rate may be due to the singular or combined effects of hypovolemia and hypertonicity (Sawka, 1992). It has been suggested that in a temperate environment the core temperature may increase by 0.10°C for every 1% loss in body weight (Greenleaf & Castle, 1971). Other studies in desert and hot environments have reported graded increases in core temperature ranging from 0.20-0.40°C for every % decrease in body weight (Sawka, 1992; Gisolfi & Copping, 1974). This makes the potential for heat illness a serious concern to athletes making weight by using dehydration practices, especially if they are exercising in a hot environment. Conversely, an investigation in which hyperhydration was induced with glycerol infusion showed an increase in hydration that lead to a decreased core temperature and increased sweating rate during moderate intensity exercise in a warm environment (Lyons, Riedesel, Meuli &Chick, 1990).

In addition to concerns for impaired thermoregulation and cardiovascular function, it has been suggested that the practice of making weight can have a negative impact on protein nutritional status, and may lead to stunted growth (Horswill, Hanpark & Roemmich, 1990; Hansen, 1978). Possible effects of dehydration on the immune system have not been clearly studied, but a study which examined the immune system in response to dehydration and attempted rehydration, the researchers found a decreased $CD4^+$/ $CD8^+$ T lymphocyte ratio and suggested that possible immunosuppression could be due to the stress of dehydration (Greenleaf et al., 1995). Hypohydration may lead to any of the potential health concerns stated previously. A further complication may be that repeated dehydration can lead to acute and chronic renal ischemia. Many wrestlers and

rowers believe that they can avoid the consequences of rapid weight loss by rehydrating themselves over the short period between weigh in and competition, but research shows that the time typically does not allow full or sufficient recovery (Burge et al., 1993; Costil & Sparks, 1973; Vaccaro, Zauner and Cade, 1976).

Psychological Effects of Hypohydration

Hypohydration may also impact the performance of an exercise task through an impact on the athletes' state of mind. Research on the effects of rapid weight loss (a combination of hypohydration and caloric restriction), has shown that the cognitive function of the athlete may be compromised (Horswill, Hickner, Scott, Costill, and Gould, 1990; Gopinathan, Pichan & Sharma 1988; Hansen, 1978). Horswill et al. (1990), observed that a combination of food and fluid restriction leading to a 6% decrease in body weight had a negative effect on selected psychological variables of increased tension, depression, anger, fatigue and confusion and lower vigour through a profile of mood states (POMS). Research has suggested that the repeated weight loss practices by wrestlers in educational settings subject may receive lower grades and have impeded social development (Hansen, 1978). Gopinathan et al., (1988) showed that with a body fluid deficit of more than 2%, a significant decrease in mental performance on a series of psychomotor tests could be observed in heat acclimated individuals.

While most research points to the problems dehydration may cause not all studies shown this negative effect. Bijlani and Sharma (1980) found that a 3% decrease in body weight by dehydration did not effect mental performance (reaction time and proof reading), while Morgan (1970) actually showed a decrease in anxiety with decreasing body weight when 5% of body weight was lost over one week. Although the research may not show complete support for impaired psychological function, any evidence for increased stress in the athlete should be cautioned due to the potential impact on health and performance.

Performance Effects of Hypohydration

It is generally accepted that a reduced body water content adversely affects performance. The level of body water deficit that is critical to induce a performance decrease and the size of that impairment has been thought to be dependent on the environment and task performed (Sawka, 1992). A hotter environment seems to increase the risk of performance impairment and prolonged aerobic tasks are more susceptible than shorter anaerobic ones (Sawka & Pandolf, 1990). The effect that hypohydration has on the performance of exercise tasks has been well studied, and while the research is somewhat equivocal, hypohydration has been suggested to impact negatively on strength, endurance, maximal aerobic power and cognitive function (Tipton, 1982, Caldwell, Ahonen, Nousiainen, 1984).

The research performed on the critical threshold at which hypohydration effects performance has shown that in a temperate (neutral) environment a bodyweight decrease of less than 3% had no effect on maximum aerobic power (MAP) (Armstrong, Costill & Fink, 1985; Caldwell et al., 1984). When hypohydration lead to a body weight decrease of more than 3%, MAP was observed to decrease in 3 studies (Buskirk, Iampietro and Bass, 1958; Caldwell et al., 1984; Webster, Rutt and Weltman, 1988). These latter studies showed decreases of MAP in the range of 4-7% with dehydration to 3-5% of bodyweight. This evidence suggests that a body weight decrease of 3% may be the critical threshold of dehydration at which MAP may be affected. In a hot environment the critical threshold seems to be lower as studies with small (2%) and moderate (4%) body weight decreases shown to result in a large decrease in MAP (10% and 27% respectively) when treadmill exercise was performed in the heat (Craig and Cummings, 1966).

Physical work capacity (PWC) for incremental aerobic exercise is decreased during hypohydration despite no change in MAP (Armstrong et al., 1985; Caldwell et al., 1984). The decrease in PWC appears to be related to amount of weight lost and was also larger when exercise was performed in hot environments. This leads to the suggestion that the thermoregulatory system (via an increased body temperature) has a role in the reduced exercise performance mediated by a body water deficit. It is apparent that

changes in distribution of blood flow, increases in blood viscosity and decreases in blood pressure may affect cardiac output and thereby influence MAP and PWC (Sawka, 1992).

A decrease in physical work capacity as measured by changes in work time at maximal intensities have been shown even when there was no impairment in MAP (Saltin, 1964a, 1964b). The effect of dehydration (3.6-4% body weight loss) by either heat stress, exercise or both on aerobic and anaerobic work capacity was examined. He found no significant change in MAP, cardiac output, stroke volume, or anaerobic capacity, but work time during a maximal cycle test decreased markedly (from approximately 6min to 4min) reflecting a decreased physical work capacity. While there are conflicting findings reported with respect to changes in MAP, the decreased work capacity or endurance time are the most consistent and important effect related to the performance of rowing exercise.

The possible impact that rapid weight loss by hypohydration could have on anaerobic power performances was reviewed by Horswill (1991). He summarizes the relevant data to suggest that despite the detrimental physiological changes, dehydration induced weight loss does not impair anaerobic power performance lasting less than 30 seconds. The research on "weight cutting" that has shown an impact on anaerobic type of performances has involved some caloric restriction over several days, and should be viewed separately from the hypohydration situation. Horswill et al., (1990) examined a 6% bodyweight loss over 4 days and the effect of a high carbohydrate and low carbohydrate diet used to achieve the weight loss. They observed some impairment of a high intensity, 6 minute intermittent sprint arm crank exercise. The high carbohydrate diet conferred some resistance to the amount of performance and psychological impairment but could not eliminate it. In a similar study McMurray, Proctor and Wilson (1991) found a decreased power output on a Wingate test due to a 3% weight loss over 7 days by caloric restriction in a normal diet group.

The impact of hypohydration on strength is difficult to assess. In a study by Ahlman and Karvonen, (1961) they report no decrease in strength with dehydration in wrestlers; and, Saltin (1964a,b) showed that isometric strength measures where no

different after dehydration than before. While in contrast, Bijlani and Sharma (1980) reported a 3% decrease in body weight by dehydration concurrent with a reduced endurance time for isometric contraction of forearm extensors. Houston et al. (1981) found a 4 day weight loss protocol (combined caloric restriction with some fluid restriction) lead to a decrease in muscle glycogen and dynamic strength measured by isokinetic knee extensor peak torque. In a case study of two wrestlers who used there own methods of weight loss (no diuretics), the researchers observed that strength measures of peak torque, time to peak, rate of peak torque development and maximum power in a single maximum contraction decreased with reduced bodyweight (5.1-5.8% over 3 days) as well as performance during a 5 minute isokinetic intermittent performance test (Oopik, Paasuke, Sikku, Timpmann, Medijainen, Ereline, Smirnova & Gapejeva, 1997). Differences in the observations may be related to the method of weight loss (ie. whether caloric restriction was involved), and the duration of the weight loss period.

Overall, it has been established that hypohydration limits performance. This limitation may have both aerobic and anaerobic components, and although the impact of hypohydration on maximum oxygen consumption, strength, and peak power may be conflicting, physical work capacity, and endurance time at maximal intensities is decreased with hypohydration.

Specific Effects of Body Fluid Loss Method

In a study that compared performance effects of weight loss (4.1% on average) by three different methods, sauna, diuretic or exercise protocols, the exercise-induced weight loss group showed less impairment of cardiovascular performance variables (Caldwell et al., 1984). This latter research observed that O_2 consumption, O_2 pulse and workload as well as blood lactate were decreased (at maximal exercise) in those who lost weight by sauna or diuresis but not the exercise group compared to controls. One problem that complicates the issue is that the sauna and diuretic groups lost weight over 24 hours, where the exercise group lost weight over 48 hours. These findings are supported by Kozlowski & Saltin (1964) who showed exercise-induced hypohydration may lead to less

water loss from the plasma . It was suggested that water released from the burning of fat and carbohydrates as fuel provides protection for the plasma volume. This would provide a rational for exercise-induced dehydration to be a superior method of weight loss.

2.8 The Physiology of Rowing

Competitive rowing involves high intensity exercise over a 2000m distance that lasts from 5.5 - 7.5 minutes depending on the size of the boat and crew. Many studies have investigated the physiological demands of on water rowing and simulated rowing tests on the Gjessing rowing ergometer and Concept II rowing machines which are used for off season training (Hagerman & Lee, 1971; Lormes, Buckwitz, Rehban & Steinacker, 1993). It is well established that aerobic and anaerobic demands are very high for rowing and the rower must have the ability to generate high muscular strength and power (Secher, 1983). Maximum aerobic power is somewhat less than the estimated net cost of rowing at racing speed and the difference between the rower's maximum aerobic power and a cost of 6.7 to 7.0 l/min over 6.5 min (approximately 21-30%) must be considered to be the anaerobic contribution (Steinaker, 1993). Hagerman (1978) suggested a 70% aerobic and 30% anaerobic contribution to a 6 min all out test at 96-98% of maximum aerobic power. With the high demand for aerobic power, it is not surprising that MAP was shown to be the best predictor of rowing performance $r=0.95$ ($p<0.05$) (Penkman & Bell, 1996). Secher (1993), also suggested the anaerobic importance to the event as one reason that heavyweights may be superior to lightweights as the former have higher anaerobic capacity. The involvement of the upper and lower body simultaneously in rowing make it a unique exercise with an extremely high physiological strain on the body. The literature briefly reviewed above demonstrates the high intensity exhaustive nature of rowing. The addition of weight cutting procedures and the resulting dehydrated state may bring about physiological consequences not observed in other acute short term exercise bouts.

2.9 Effects of Rowing on the Immune System

Immunology research has been performed using many different exercise modes, including running, cycling, triathlon, basketball, and swimming. Compared to other sports, there has been relatively little research published with rowing used to provide the exercise stress. Early evidence suggested that rowers might be at risk to immune suppression due to a decreased eosinophil count even in anticipation of the Harvard-Yale 4 mile boat race (Renold, Quigley, Kenard & Thorn, 1951). A more recent investigated the response of the immune system immediately following exhaustive exercise in 8 rowers, and found an increase in total lymphocytes, although it is not clear if this was a bike or rowing test (Werle, Jost, Koglin, Weib and Weicker, 1989; in Weicker and Werle, 1991) This increase was associated with a decrease in $CD3^+$ (T) and $CD4^+$ cells and a relative increase in $CD8^+$ lymphocytes resulting in a decreased $CD4^+/CD8^+$ (helper/suppressor) ratio. This ratio is often used as an indicator for immunosuppression, but in this case the authors were hesitant to conclude that this alteration in T and B cell systems expressed impairment or amelioration of the immune system or only a physiological response. Recently, Neilsen et al. (1996), showed no immunosuppressive action of 6 minute all-out ergometer rowing in heavy weight male rowers (n=10). The counts of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$ lymphocytes and NK cells ($CD16^+$) increased during exercise. The $CD3^+$ and $CD8^+$ lymphocytes decreased to below pre-exercise values two hours after exercise, while the LAK and NK cell activity increased. A second study by these same researchers examined the effect of repeated bouts of the exercise over a two day period (2 x 3 bouts of 6 minute ergometer exercise) on eight male oarsmen. This work resulted in even higher values for NK cells, NKCA, leukocytes neutrophils, lymphocytes (a wide range) and monocytes than the single exercise bout. The values remained elevated during the day of recovery following the second exercise bout (Neilsen, Secher and Pedersen, 1997). The researchers suggested that the data provide no indication of any immunosuppression due to the rowing exercise, but stated that a change in the susceptibility to infection would provide more information. This research investigated only one sub-population of the rowing community, the heavyweight male oarsmen. In light of the different physiological strains placed on the lightweight

rower trying to make weight, and evidence that the female's immune system may respond differently, these results should not be generalized to other rowers without further study.

2.10 Clinical Incidence of Illness in Rowers

As has been demonstrated, long duration, exhaustive endurance exercise is the only situation where a sustained depressed immune function has been found *in vitro*, and related clinically to an increased incidence of illness in those athletes performing this type of exercise. The clinically relevant information to exercise in general was reviewed earlier. In this section of the review of literature, epidemiological and clinical data related to the rowing athlete will be discussed which suggests that the rowing athlete may be experiencing similar immune dysfunction as the long endurance athlete.

A recent study performed by Castell, Poortmans and Newsholme (1996) provides evidence that rowers as an athlete group may have an illness incidence rate equal to or greater than that of marathon runners. They examined a large number of both male and female athletes (n=201), from different sports (rowing, mid-distance, marathon and ultra marathon runners) during training or competition and monitored illness over the seven days following training or competition using a questionnaire. The responses indicated an illness rate of 54.5% for rowers, vs. 24.7%, 46.8% and 48% for mid-distance, marathon and ultra marathon runners respectively.

There are two additional studies performed on rowers that support the existence of a possible immunosuppression. These two studies were both performed on male rowers with no distinction of whether they are lightweight or heavy, making the application to lightweight female athletes a tenuous one. Budgett and Fuller (1989) performed a retrospective study on 69 oarsmen trying out for the British national team in 1987. The study investigated the types and rates of illness and injury over the previous year. Illness accounted for 448 lost training days while injury accounted for 884 lost days. 50 of the athletes (73%) missed training due to illness and the majority of those cases (41) were URTI. It was unclear how these rates compared to controls but a decrease in the rate of

illness would allow for increased training time, and improved performance.

A second study compared 61 university crew athletes to 126 unconditioned ROTC cadets in terms of frequency and severity of specific URTI symptoms using a validated symptoms checklist (Douglas and Hanson, 1978). The crew athletes exhibited increased frequency and severity of selected symptoms of URTI, saw a doctor and missed more classes than controls.

The three studies reviewed above provide evidence to suggest that rowing is a sport in which immunosuppression is likely evident and is a major problem accounting for lost training days, although the experimental evidence to date of the *in vitro* immune response to exercise has not provided strong evidence of immunosuppression.

2.11 Rowing Specific Performance Effects of Weight Loss

There have only been two studies that have examined the performance consequences of dehydration in lightweight rowers. Burge, Carey & Payne (1993) showed a performance decrement in lightweight male rowers (n=8) following weight loss through food and fluid restriction with light exercise. This weight making protocol resulted in dehydration (5.16% decrease in body weight lead to a 12% decrease in plasma volume), and a two hour rehydration period did not allow for complete rehydration. The performance test was a maximal rowing trial on the Gjessing rowing ergometer (4200 revs, 3kg resistance). The average trial time increased significantly from 7.02± 0.17 min for the euhydrated trial (ET) to 7.38± 0.21 min for the rehydrated trial (RT) ($p<0.05$) The magnitude of the performance decrease was related to the level of dehydration indicated by the change in plasma volume remaining after rehydration ($r= 0.93$, $p<0.01$). The thermoregulatory response to this treatment was not investigated. This study demonstrates the severe rowing performance consequences that may result from attempts to cut weight which result in hypohydration.

Koutedakis, Pacy, Quevedo, Millward, Hesp, Boreham and Sharp (1994) compared a two month weight reduction period to four months and the impact on selected performance parameters. While lightweight females were the subjects of this study, the

weight reduction period was relatively long, and involved caloric restriction limiting its application to the rapid weight loss situation. In general, this study showed that the 4 month weight loss period, was more beneficial than the shorter two month period in terms of impact on performance parameters such as VO_2max, and recommended that weight be reduced over as long a period as possible.

These studies show the limited information available on the consequences of a rapid weight reduction through hypohydration on the lightweight female rower. The study by Burge et al. (1993) provides a model for the dehydration rehydration protocol and the exercise performance test is similar to the 2000m Concept II rowing time trial, used by more athletes in North America.

2.12 Summary

It has been shown that while many studies have been performed in the area of exercise immunology, and the effect of prolonged endurance exercise well studied, acute exercise has shown varying results depending on the type of exercise mode used. Despite this, the literature does not show clear evidence of immune system impairment on *invitro* assays of peripheral blood in rowers, there is some evidence that the rower as an athlete group may be equally as susceptible to illness as the ultra marathon runner. This evidence suggests that a more clear understanding of the response of the immune system to rowing exercise is needed. The unique physiological and thermoregulatory stress that rowing in the hypohydrated state places on the body, and the suggestion that the immune system of the female is functionally different from the male, make the female lightweight rower unique. The immune responses to intense rowing exercise may be markedly different in these athletes than that observed in the literature to this point.

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CHAPTER 3

THE PHYSIOLOGICAL AND PERFORMANCE EFFECTS OF SIMULATED RACE ROWING ON FEMALE LIGHTWEIGHT ROWERS IN A EUHYDRATED AND HYPOHYDRATED STATE

Introduction

Changes in the appearance of immune cells in peripheral blood following exercise was first observed nearly a century ago (Larabee, 1902). Since then, a common belief has developed that while moderate exercise can be beneficial to the immune system, exhaustive exercise can be immunosuppressive (MacKinnon, 1992; Smith, 1995) yet this has proved difficult to define clinically. Over the past decade, the desire to better understand the influence of exercise on immune function and gain maximum benefits from training has fuelled an increasing number of investigations in this area. Researchers have performed detailed studies to investigate the effect of varying intensities, durations and modes of exercise on a myriad of immune parameters and to answer general to more specific questions. Exercise has been shown to induce changes in leukocyte subpopulations (Kettler et al., 1996; Gannon et al., 1997; Nieman et al., 1989; Bury et al., 1996), cytokine or immunoglobulin secretions (Hoffman-Goetz & Pedersen, 1993; Tomasi et al., 1982), proliferation of lymphocytes (Field et al., 1991; Nieman et al., 1995; Hinton et al., 1997; Nielsen et al., 1998), killing activity of natural killer cells (Shek et al., 1995; Nielsen et al., 1996; Nieman et al., 1995) and both phagocytic and oxidative burst function of neutrophils (Rincon, 1994; Bury & Pirnay, 1995; Suzuki et al., 1996; Lotzerich et al., 1997). Several immune responses are biphasic in nature, whereby the parameter may increase during exercise only to decrease in recovery sometimes to a value below those observed pre-exercise (Eichner, 1993). This observation has lead to the proposal of mechanisms such as the "open window hypothesis" to explain how an altered

immune response to exercise may lead to an increased chance of illness (Pedersen and Ullum, 1994).

Efforts have been made to characterize both the cell trafficking and functional responses of immune cell subpopulations in the blood compartment. Researchers have had difficulty reaching a consensus, but it appears that exhaustive endurance exercise can exert an immunosuppressive influence on the function of the innate immune system such as depressing natural killer cell activity for several hours post exercise (Shek.et al., 1995; Nieman et al., 1995). The response of the adaptive branch and the impact of shorter duration activities is less clear (Nieman, 1997). Another difficulty has been to establish a clear link between clinical observations of altered immune function and an observed reduction in resistance to illness, or vice versa. Epidemiological studies have attempted to provide this link, but a consensus is difficult to reach. An exception is the case of the marathon or ultramarathon runner where two detailed studies have provided strong evidence that the incidence of upper respiratory tract infections (URTI) are increased following competitive events (Peters and Bateman, 1983; Nieman et al., 1990).

Earlier research focussed on running, but recently studies have been done on team sports, cycling, swimming and rowing (Benoni et al., 1995; Field et al., 1991; Kono et al., 1988 ; Nielsen et al., 1996). Rowing is a sport in which the impact of the immune system is not well understood. The competitive performance is short duration and high intensity and to date clinical studies have documented little evidence of impaired immune function (Nielsen et al, 1996). In contrast, rowing has been reported to have one of the highest rates of illness among athletes of different sports including marathon and ultramarathon runners (Castell, Poortmans and Newsholme, 1996).

In 1996, a new weight category for rowing was added at the Olympic games that had existed for decades in international and domestic competition. This weight category moved rowing into the world of weight restrictive sports. As a result, new physiological challenges arose that had previously been ignored in the literature on this sport. Lightweight rowers at the club and national level have been known to adopt unsafe eating practices and try to reduce weight using short term dehydration to qualify to compete in a

lighter weight category. In contrast to the handful of studies in rowing, extensive research has been done to determine the effects of weight reduction activities on health and performance in the sport of wrestling. The potential health risks of this activity have been detailed in the ACSM position stand on weight loss in wrestlers (1978; revised 1996) and include impaired thermoregulation, immune function, hormonal status, protein nutritional status, growth and development, psychological state and others. The fact that more women are becoming involved in rowing as a competitive sport and exceed the number of men participating in Canada point to a need for new research into how these potential harmful activities might affect this understudied population of athletes in this intense sport.

Rowers may have a higher than average susceptibility to illness (Castell et al, 1996; Douglas & Hansen, 1978), yet to date no clinical evidence of immunosuppresion has been documented in these subjects (Nielsen et al, 1996; 1996; 1998). Dieting in obese subjects and short term weight loss in female athletes have been shown to be immunosuppressive (Kelley et al., 1994; Field et al., 1991; Kono et al, 1988); hypohydration is thought to contribute to the immunosuppressive response to spaceflight (Greenleaf et al, 1995; Sonnenfeld, 1998); and the combination of mental, physical and general stress may have an additive effect upon the immune system (Hoffman-Goetz and Pedersen, 1994). This evidence suggests that the lightweight female rowers may combine several potentially immunosuppressive stressors in one competitive event, yet this situation has not been investigated to date. Therefore the purpose of the study was to investigate the effect of a 24 hour weight cutting (fluid restriction) protocol followed by the performance of a 2000m simulated rowing test on the peripheral immune cells and their function. Furthermore, the effect of the weight reduction on the performance on the 2000m rowing test and thermoregulatory responses were studied. It was hypothesized that the performance of a 2000m rowing test in a hypohydrated state would result in an impaired immune response indicative of immunosuppression. In addition it was hypothesized that this exercise performance in a hypohydrated state would result in impaired performance and thermoregulation when compared to the euhydrated state.

Methods

Subjects

Seven healthy female rowers (age 22.4 ± 3.6 yr, weight 61.1 ± 3.4 kg, height 164.4 ± 3.0 cm; mean ± SD) from the Edmonton area volunteered for the study, which was approved by the ethics board of the Faculty of Physical Education and Recreation at the University of Alberta. Six subjects had at least one previous season of rowing experience, while one was a novice (experience 2.6 ± 2.1 yr). All subjects completed a demographic questionnaire (Appendix A) and signed an informed consent after the purpose and procedures of the study were explained (Appendix B).

Experimental Design

The study used a randomly assigned, within subjects, two conditions (euhydrated, hypohydrated) experimental design to determine the effects of a weight restriction protocol on performance and physiological variables. Each subject performed two 2000m maximum effort rowing trials; a normal or euhydrated trial (ET) and a trial that followed a standardized dehydration and rehydration protocol (RT) (n=7). A random cross-over design was used to assign the treatment order. This resulted in 3 subjects completing the RT followed by the ET and 4 subjects completed the testing in the reverse order. The two trials were performed at the same time of day (8:00 am - 12:00pm) and were separated by 72 hours to try to provide adequate recovery between the two 2000m trials and to limit potential for cyclic menstrual hormone changes influencing the immune response. Due to scheduling conflicts, however, two subjects received a further 48 hours of recovery between the two testing sessions. We were unable to control for menstrual phase of the subject, or oral contraceptive use; however, the menstrual status of each subject was assessed by questionnaire (Appendix A) in order to provide an indication of the timing of the cycle. Two subjects were oral contraceptive users while 5 were not. Of the 5 non-oral contraceptive users, three subjects were tested during the follicular phase (actual testing days ranged from day 7-14) while two were tested during the luteal phase (actual testing

days ranged from days 20-25). Of the two oral contraceptive users, one subject was tested between day 19-22 and the other between day 11-16. Not controlling for the phase of menstrual cycle is acknowledged as a limitation in the design of the present study. To date, experimental evidence has not shown any clear impact of menstrual cycle timing on the immune response to exercise but further research is necessary to verify this phenomena (Northern et al., 1994; Zelazowska et al., 1997).

The weight restriction treatment was similar to the dehydration / rehydration protocol of Burge et al. (1993). Subjects were required to lose 3.5 % of their baseline body weight (established by morning weigh in for three days prior to the experiment) over the 24 hrs prior to the performance test for the hypohydrated trial. This weight loss was accomplished by a combination of fluid restriction with light exercise in sweat gear and overnight fast. The subjects were asked to monitor the progress of their weight reduction over the 24 hour period on a scale provided. The subjects reported to the lab on the morning of the test and were weighed using the same scale to determine if the target body weight had been reached. Those who reported to the lab and had not lost the required (3.5%) body mass were asked to perform light exercise in sweat gear and were weighed periodically until the target mass was reached. Subsequently the subjects were given a 2 hour rehydration period during which they were given 250 ml of water every 15 minutes and re-weighed. Immediately following the two hour rehydration period the subjects performed an all-out 2000m simulated rowing test. Serial blood samples were taken pre-dehydration (24 hours prior to dehydrated weigh in), at weigh-in (post-dehydration), pre-rowing test (post-rehydration), immediately post erg-test and following 1 hour of recovery. See the time line below:

↓ ----24hr fluid restriction---- ↓ ----2hr rehydration—— ↓ ---2000m test--- ↓ ---recovery--- ↓
(light exercise/sweat) CII rowing (1hr)

weigh in 24 hr pre test blood #1	8:00am weigh in blood #2	10:00am weigh in (rehydr.) blood #3	post test blood #4	recovery blood #5

The euhydrated trial followed the same experimental design, except no weight reduction was required. A 9:00 am weigh-in and 30 min. rest period was adhered to before the test.

Assessment of Performance

Two performance tests (ET and RT) were conducted on the Concept II model C rowing machine (Concept II, Morrisville, VT.) consisting of rowing 2000 metres as fast as possible simulating a rowing race. Total time (in seconds) to complete 2000m was recorded. The rowing test was performed following a 10 minute warm up and subjects attested to having refrained from intense training in the 24 hr prior to the test.

Assessment of Thermoregulatory Response

Tympanic temperature was measured using the Thermoscan® infrared thermometer (Braun, USA). Measurements were made by inserting the head of the thermometer into the ear canal of the subject immediately prior to each blood sampling time point. All measurements were taken by the same individual.

Analytical Methods

Blood Collection and Hematological Analysis

Blood samples were taken by a registered nurse from the back of the forearm or hand using an indwelling catheter kept viable with 0.5% saline solution. If the indwelling catheter did not remain viable or caused the subject too much discomfort, repeated venipunctures were used. Four blood samples were taken during each testing session: 1. at weigh in (3ml); 2. pre-test (10ml); 3. immediately post-test (10ml); and after one hour of recovery (10ml). The 10 ml samples consisted of 3ml collected in an EDTA treated vaccutainer (purple top, Becton-Dickinson, Mississauga, ON.) for CBC analysis and 7ml collected in a heparin treated vaccutainer (green top, Becton-Dickinson, Mississauga, ON.) for detailed immune analysis. All testing was performed between 8:00am and 1:00 pm. Analysis was performed on blood immediately following the

completion of the exercise testing session. Early blood samples were immediately placed on ice for the duration of the testing session. Complete blood counts, using a Coulter STKS instrument (Coulter Electronics, Inc., Hialeah, FA) and manual differential determinations were performed on all samples by the staff of the Hematology Laboratory at the University of Alberta Hospital.

Mononuclear Cell Phenotyping

100 μl of whole blood was added to wells of a microtiter plate. Red blood cells were lysed using warm lysis buffer and immune cell subsets characterized by immunofluorescence assay using monoclonal antibodies (MAb) specific for the different human immune cell subsets (Sigma, St. Louis, Missouri). MAb's were conjugated to one of the following flourescent markers: biotin (B), phycoerythrin (PE) or fluorescein isothiocyanate (FITC). Single labelling of CD3-B T cells (associated with TCR alpha, beta, delta and gamma), CD4-FITC helper/inducer T cells (T4, co-recognition of MHC class II with TCR), CD8-B cytotoxic/suppressor T cells (NK subset T8 co-recognition of MHC class I with TCR), CD14-FITC monocytes (granulocyte, macrophage, dendritic cells and B cells), CD16-PE NK cells (granulocytes, macrophages and monocytes) and CD20-PE B cells (precursor B cell subset) was performed. CD4-FITC and CD8-B were also analyzed double labelled with CD25-PE (activation marker, IL-2 receptor analog). All MAb's were diluted in phosphate buffered saline at pH 7.4 containing bovine serum albumin (fraction V) (Sigma, St. Louis, Missouri) and 0.1% (w/v) sodium azide. Appropriate isotype controls were used to account for nonspecific binding of the monoclonal antibodies. Whole blood depleted of red blood cells was incubated for 30 minutes at 4 °C with antibodies, washed twice in 200 µL of phosphate buffered saline containing fetal calf serum (40 g/L), and incubated for another 30 minutes at 4 °C with the streptavidin conjugate in wells contained biotinylated MAb. Cells were washed twice and fixed in phosphate buffered saline containing paraformaldehyde (10 g/L plus Na azide) and analyzed on a FACScan® (Becton-Dickinson, Sunnyvale, CA) according to the relative fluorescence intensity. Analysis was done after the lymphocytes were gated based

on forward and side scatter characteristics. Resulting percentages were corrected for background fluorescence (0-5%) determined by incubating the cells with isothiocyanate-conjugated goat anti-mouse IgG only.

An estimation of absolute concentrations of the different subpopulations of lymphocytes was obtained by multiplying the relative concentrations determined via immunofluorescence by the concentration of lymphocytes determined from the CBCs.

Preparation of Lymphocytes

Lymphocytes used for proliferation were isolated from whole blood using Ficoll - Hypaque gradient (1077) (Sigma, St. Louis MO.) centrifugation at 1840 rpm for 30 min. After gradients were removed, the cells were washed three times with Krebs- Ringer HEPES (KRH) buffer with bovine serum albumin (5 g/L). If red blood cells remained in the fraction they were lysed using sterile lysis buffer + anibiotic/antimytotic (Gibco, Burlington, ON.) for 5 minutes, pelleted and washed (2x). Cells were prepared and cultured in RPMI (Fisher Scientific, Edmonton, AB.) supplemented with fetal calf serum (50 g/L), 2.5 μmol/L 2-mercaptoethanol, 4000μmol/L glutamine, penicillin (100 units/mL), streptomycin (100 μg/mL) and HEPES (25 mmol/L).

Mitogenic Responses of Immune Cells

The isolated lymphocyte solutions were counted and diluted to a concentration of (1.0×10^9/L) in the media described above. 200 μl of the solution was cultured in 96 well microtiter plates without mitogen or with either Concanavalin A (Con A; 5 mg/L) (ICN, Montreal, PQ.) or Phorbol Myristate Acetate (PMA; 40μg/L) (ICN, Montreal, PQ.) and incubated for 48 hours. Cells were incubated in humidified 5% CO_2 atmosphere at 37°C. Eighteen hours before harvesting the cells, each well was pulsed with of [^{3}H] thymidine (18.5 kBq) (Amersham, Oakville, ON.). Cells were harvested on glass fibre filters using a multiwell harvester (Skatron, Lier, Norway) and counted using Ecolite® in a Beckman betacounter (LS 5801®,Beckman Instruments Inc., Missisauga, ON, Canada). All assays

were performed in triplicate. The lymphocyte proliferative response was measured by the incorporation of tritiated thymidine into the DNA of a sample of cells following stimulation with a mitogen. A liquid scintillation counter was used to determine counts per min and stimulation index (SI) was determined by comparing the response of the stimulated cells to that of the non stimulated cells through the following formula: SI= ([^{3}H] thymidine (counts/min) incorporated by stimulated cells)-([^{3}H] thymidine (counts/min) incorporated by unstimulated cells)/ ([^{3}H] thymidine (counts/min) incorporated by unstimulated cells).

Neutrophil Oxidative Burst

Neutrophil oxidative burst was carried out using 400 μl of whole blood depleted of red blood cells as previously described (Vowells et al., 1995). After a 5 minute incubation with dihydrorhodamine-123, 100 μl PMA (ICN, Montreal, PQ.) was added to reaction tubes which were further incubated and an aliquot removed at 5, 10 or 15 minutes and immediately placed on ice to stop the reaction. The oxidation of dihydrorhodamine-123 to rhodamine-123 was immediately analyzed by flow cytometry (CellQuest, Becton Dickenson). Neutrophils were gated according to their forward and side scatter characteristics. Mean channel fluorescence of gated neutrophils was measured at 0, 5, 10 and 15 minutes. SI = (Mean channel fluorescence time B) - (Mean channel fluorescence time A) / (Mean channel fluorescence Time A) .

Statistical Analysis

Standard descriptive statistical methods were used to calculate means and standard deviations. A two-way (trial and time) analysis of variance (ANOVA) with repeated measures was used to determine potential differences in immune cell counts and tympanic temperature between the ET and RT at weigh in, immediately pre-test, post test and recovery. A 2 x 3 ANOVA with repeated measures was also used to determine differences between the trials and testing time point for the flow cytometric analysis of immune cell subpopulations, lymphocyte function and body weight. The neutrophil

functional assay was analysed using a 2 x 3 ANOVA with repeated measures (trial and time) at each of the assay time points (0, 5, 10 and 15 min). A 2 x 4 ANOVA was used to determine differences between the assay time points for neutrophil function. A Newman-Keuls multiple comparison procedure was used to assess any main and/or interaction effects revealed by the ANOVA's. The alpha level was set a priori at $p<0.05$. The difference in performance between the ET, RT and the testing order was determined by a t-test with alpha level set a priori at $p<0.05$. All statistical analyses were conducted using the Statistica software package (Version 6, STATSOFT, Oklahoma City, OK).

Results

Weight Loss

The 24 hour period of fluid restriction was combined with light exercise before the weigh-in for all subjects. At weigh-in body mass significantly decreased 3.33±.14% from baseline to a mass of 59.11±3.26 kg and was significantly different than all other weigh-ins ($p<0.05$, Table 3-1). The two hour rehydration period allowed the subjects to replace 1.74±.36 kg of the mass lost to 60.85±3.44 kg which was not different than baseline. ET weigh-in was not significantly different than baseline.

Performance

The time taken to complete the 2000m simulated rowing test was recorded in seconds for the ET and the RT. The mean time to complete the ET was 487.29±18.56 s and the mean time to complete the RT was 487.57±18.80 s. No significant difference was observed between the two trials. The tests were also compared for the order of the testing sessions. The mean performance time for the first and second test was 486.19± 17.55 s and 488.67±19.65 s respectively. Again no significant difference was noted when the order of the trials were compared.

Tympanic Temperature

The tympanic temperature of the subjects changed significantly during the testing session and showed significant differences between trials (Fig. 3-1). Following the performance of the exercise bout in the ET the temperature increased from 35.5 ± .46 °C pre-test to 35.96 ± .55 °C ($p<0.05$). This measurement returned to the pre-test value by one hour of recovery. During the RT all temperatures were significantly higher than during the ET ($p<0.05$). The temperature increased post-test during the RT to 36.53±.31 °C, significantly higher than all other measurements ($p<0.05$). During recovery the temperature was not different than the RT pre-test value, but was significantly higher than the recovery value for the ET ($p<0.05$).

Immune Measurements

Complete Blood Cell Counts

The CBC analysis of the blood samples revealed both a main effect for time and an interaction effect for time and trial. A consistent leukocytosis was observed following exercise for both the ET and RT (Table 3-2). The white cell concentration increased from pre-test levels of 4.24±0.74 x 10^9 and 5.68 ±0.75 x 10^9 cells/L (for the ET and RT respectively) to 8.64 ±1.22 x 10^9 and 13.16±1.16 x 10^9 immediately post exercise ($p<0.05$). The post-test RT value was significantly higher than all other values but decreased to a value not different from the pre-test value after one hour of recovery (Fig. 3-2). During the one hour recovery period the WBC count of ET remained elevated when compared to pre-test values ($p<0.05$) The leukocytosis consists of a neutrophilia and lymphocytosis. Significant differences were observed across the two trials and sample points. The lymphocyte counts increased 3 fold from pre-test to post-test values in both the ET and RT (1.66±0.30 x 10^9 to 4.56±1.03 x 10^9 and 1.74±1.04 x 10^9 to 5.30±1.90 x 10^9, $p<0.05$) (Fig. 3-3). This lymphocytosis was followed by a reduction during the one hour recovery period with the recovery values not significantly different that pre-test

values.

The neutrophil counts revealed a different response between the two trials (Fig. 3-4). During the ET a delayed neutrophilia was observed as the concentration of neutrophils increased slightly during the test from a pre-test value of 2.02±0.58 x 10^9 and increased further during the recovery period to a value of 5.58±2.71 x 10^9, almost three fold higher than pre-test values ($p<0.05$). The RT resulted in a neutrophil concentration in the peripheral blood immediately following the exercise bout of 6.84±1.79 x 10^9 cells/L, significantly higher than the ET pre-test and post-test values. There was no further increase over the one hour recovery period for the RT and the recovery value decreased slightly but was not significantly different than the post-test value.

Immunophenotyping of Lymphocyte subpopulations

The exercise treatment lead to major shifts in the immune cell subpopulations from pre-test to post-test. The shifts in relative populations of lymphocyte subsets did not show any differences between the ET and RT (Table 3-3). A decrease in the relative populations of $CD3^+$, $CD4^+$, $CD4^+CD25^+$ occurred from pre-test to post-test as did the $CD4^+/CD8^+$ ratio ($p<0.05$). This relative decrease was accompanied by a significant increase in the percent of $CD8^+$ and $CD16^+$. The $CD16^+$ value increased from 10±4% to 33±3% for the RT and from 12±7% to 28±10% for the ET ($p<0.05$). The relative populations of $CD14^+$, $CD20^+$ and $CD8^+CD25^+$ were not detectable. Following one hour of recovery, all changes in relative concentrations of lymphocyte subpopulations returned to values not significantly different from the pre-test.

The estimation of absolute concentrations of these lymphocyte subpopulations demonstrates consistent increases in most cell types immediately post exercise. The post exercise lymphocytosis was large enough to produce significant elevations in $CD3^+$, $CD4^+$, $CD8^+$ and $CD16^+$ lymphocytes ($p<0.05$; Table 3-4). Analysis of these responses revealed a main effect for time with no significant interaction effects between the trials. This post exercise increase in lymphocyte subpopulations was reversed as the lymphocyte concentrations decreased during recovery. All lymphocyte subpopulations had decreased

to a value not different from pre-test values with the exception of $CD4^+$. The $CD4^+$ cells decreased to 0.57±0.14 and 0.46±.13 $x10^9$ cells/L for the RT and ET respectively, significantly less than the pre-test and post-test values ($p<0.05$). The double labelled $CD4^+25^+$ cells did not increase significantly during the test, but the recovery value was significantly decreased with respect to the post-test value. The total response of the $CD16^+$ cells showed a main effect for trial as well as time and was greater for the RT compared to the ET ($p<0.05$).

Lymphocyte proliferation (activity)

When examining the raw counts per minute (cpm) for the unstimulated lymphocytes, no significant effects were observed between the two trials or times within each trial (Table 3-5).

PMA stimulation

During both trials the 24 hour stimulation index of the lymphocytes in response to simulation with PMA decreased significantly from pre-test values of 67±59 and 40±15 (RT and ET respectively) to 27±26 and 12±8 immediately post-test ($p<0.05$, main effect for time) (Fig. 3-5). During the one hour recovery period the stimulation indices returned to values not significantly different from the pre-test. There were no significant differences between the two trials. The raw cpm also did not demonstrate any significant differences between trials, but did show a main effect for time as the recovery values were significantly increased from the pre-test and post-test values ($p<0.05$) (Table 3-5).

ConA stimulation

The proliferative response of lymphocytes in response to stimulation with Con A was similar to the response to PMA stimulation in that a main effect for time was detected (Fig. 3-6). The stimulation index measured immediately post-test was again depressed from the pretest values for both trials (26±16 and 23±10 post-test for RT and ET respectively compared to 99±64 and 97±36 pre-test). Following one hour of recovery however, the stimulation index for the had not fully recovered. The stimulation index at 1

hr of recovery was still significantly less than the pre-test value ($p<0.05$). The raw cpm however, did not demonstrate any significant effects.

Neutrophil Oxidative burst activity

There were significant increases in mean channel fluorescence of neutrophils in response to stimulation with PMA, observed from 0-5 min, 5-10 min and 10-15 min time points ($p<0.05$). This was a consistent observation for all time points (pre, post, recovery) and both trials (ET and RT) with no differences existing between trials (table 3-6). The ratio of increase, represented by the stimulation index indicates that the 0-5 min stimulation index is significantly greater than the 5-10 and 10-15 min SI for the pre-test and post-test samples ($p<0.05$; Table 3-7). For the recovery sample the 0-5 min SI was significantly different from the 10-15 min SI only ($p<0.05$; Table 3-7)
When comparing the neutrophil activity of the blood samples between trials and across sample points of the testing at the same assay time point, significant differences where observed between the blood sample time points, but not between the two trials. At the 0 and 5 minute time point of the assay, no significant effects were observed. At the 10 and 15 minute time points of the assay however, a main effect for time was detected. Post-hoc analysis revealed that the post-test mean channel fluorescence indicative of activity of the neutrophils was significantly lower than the pre-test or recovery values ($p>0.05$), while the pre-test and recovery values were not different from each other.

Discussion

It was hypothesized that the 24 hour weight restriction protocol followed by the performance of an all-out 2000m simulated rowing test on the Concept II rowing machine would result in an impaired performance, a higher level of heat stress and an altered immune response when compared to the same test performed in a euhydrated state in female lightweight rowers. The findings of this study do not support the hypothesis that

performance would be compromised. Body mass was reduced by 3.33% through fluid restriction and exercise induced sweating with no significant difference in mean performance times between the RT and ET. The results do, however, support the hypothesis that heat stress would be greater in the rehydrated trial as tympanic temperature measurements were significantly greater during RT compared to ET. Observations that shifts in the concentrations of neutrophils and the relative concentrations of the $CD16^+$ lymphocyte subpopulation in peripheral blood differ between the two trials provide some evidence of an altered immune response. The results of this study did not support any significant functional immune suppression during the RT that was different than the ET, although the immune response to the rowing performance was different than previously observed in men (Neilsen et al., 1996; 1998). While not conclusive, indicators such as depressed lymphocyte proliferation, decreased post-test neutrophil oxidative burst despite large increases in numbers and a reduction in $CD4^+$ and $CD4^+$ 25^+ lymphocytes in recovery may indicate a compromised immune response.

The 24 hr fluid restriction protocol resulted in a significant reduction in body mass. The "weight cutting" protocol was similar to that used by Burge et al. (1993) with the exception that the subjects were not required to exercise the evening prior. All subjects chose to perform a pre-testing light exercise induced sweating session to reach the target body mass. This is closer to the sequence of events they would perform in an actual race setting. The significant reduction in body mass (2.03±0.15kg) was less than that lost by the male subjects in a similar study (Burge et al., 1993). This reflects the smaller mass of these female subjects and the modification made to the protocol in which 3.5% was the target body mass loss. The two hour rehydration period, during which the subjects ingested 250 ml of water every 15 min, recovered body mass to a value not significantly different than baseline or ET weigh-ins. This was the same protocol used by Burge et al.,(1993) based on research from Lamb and Brodowicz (1986) suggesting that this potentially allowed for optimum reabsorption.

The fluid restriction protocol in the RT did not result in a significant decrement in performance compared to ET as measured by the 2000m rowing test. This is in contrast to

the decreased rowing performance observed by Burge et al., (1993) and contrary to what was hypothesized. A 3.33% decrease is beyond what is reported to be the critical line at which reduction in body mass by dehydration leads to a decrease in VO_2max and impaired performance (Buskirk et al., 1958; Caldwell et al., 1984; Webster et al., 1988). This can be accounted for in the present study by the rehydration period. Compared to the subjects in Burge et al. (1993), the female subjects in the present study are smaller and reduced their weight less (3.33%, 2.02kg vs. 5.15%, 3.78 kg). The 2 hr rehydration period therefore allowed time for a relatively greater fluid level replenishment in the present study. We can estimate that 93.6% of the 2.02 kg of weight lost was water (Costill et al., 1976), equivalent to 1.87 kg. The approximately 1750 ml of fluid ingested appears to have replenished this body mass loss to a level no longer sufficient to impair performance.

While performance time in the RT was not impaired despite the rapid reduction in body mass, the thermoregulatory response did suggest an increased thermal stress compared to ET. Both trials resulted in a significant elevation in temperature after the exercise bout that recovered to pre-test value within one hour of recovery. However, the tympanic temperature measured at all time points during the RT was higher than ET, with the post-test value in the RT significantly higher than all other measurements. The observed 0.3 to 0.5 °C increase in temperature is supported by the literature as a 0.1 to 0.2 °C increase in core temperature is reported to occur for each % decrease in body mass (Greenleaf & Castle, 1971). While the observed temperature changes in the present study were not at a level where heat illness was a concern, if this type of activity is undertaken outside of the controlled lab setting the risk could increase. In addition, a hot humid environment can decrease the critical level of body mass reduction at which maximum aerobic power and performance may be effected (Sawka & Pandolf, 1990).

The high-intensity 2000m rowing exercise induced a strong leukocytosis in both trials. This leukocytosis, consisting mainly of a lymphocytosis and neutrophilia, was larger in the RT than the ET. The counts increased 2-fold and almost 3-fold for the ET and RT respectively. Following 1 hr of recovery in the RT leukocyte count decreased to a

value not significantly different the pre-test value. During recovery in the ET however, the leukocytes remained elevated due to a delayed increase in neutrophil counts in this trial. The post-exercise leukocytosis is the most consistent observation throughout exercise immunology literature (Nieman, 1997). The biphasic response (increasing post-test and decreasing in recovery) has been observed in studies on various immune parameters following rowing (Nielsen et al., 1996 & 1998; Werle et al., 1989 in Weicker &Werle, 1991) and other modes of exercise (Field et al., 1991; Nieman et al., 1995). This is however, the first study to compare the immune cell trafficking response between two exercise trials with a short term weight reduction treatment. Therefore the observed differences between the two trials are unique.

The lymphocyte count increased during the 2000m rowing bout in both trials to a post- test value that is 2 fold higher than pre-test and within 1 hr of recovery decreased to a value not different than pre-test. The observed shift in lymphocyte counts has been documented in the literature in other modes and intensities of exercise (Hinton et al., 1997; Kettler et al., 1996). In studies on rowing exercise the lymphocyte count increased during exercise, but decreased below pre-exercise values during recovery of 1 hr (Nielsen et al., 1996)) or 1 and 2 hr recovery (Nielsen et al., 1998). No measurements were made on lymphocyte function however.

While the lymphocyte response was similar between the two trials, shifts in the neutrophil count revealed a main effect for time, trial and an interaction effect between the RT and ET. The delayed neutrophilia observed during the ET where counts peaked in recovery is characteristic of the responses reported in the literature (Suzuki et al., 1996; McCarthy & Dale, 1988; Kettler et al., 1996; Hoffman-Goetsz and Pedersen, 1994). During the RT the recruitment of neutrophils into the circulation occurred sooner. The neutrophil count was observed to peak immediately post-test and declined during the one hour of recovery to a value not significantly different than the pre-test value.

The changes in the trafficking of immune cells do not provide direct evidence of immune suppression. The difference in the kinetics of the neutrophil recruitment between the two trials is however, a significant observation that is worthy of discussion. The

disparity in the response between the two trials can be attributed to the sequence of events in the weight cutting protocol. Cortisol has been reported to be the main stress hormone influencing the shifts in concentration of neutrophils in the peripheral blood (McCarthy &Dale, 1988), it is typically slow to rise during exercise and peaks during recovery (Pedersen et al., 1997). The 60 - 100 minutes of light exercise required to lose the weight likely resulted in a different cortisol response than what would occur in the ET. An earlier release in cortisol could lead to a higher demargination of neutrophils into peripheral blood and elevated neutrophil counts immediately following the exercise bout. This theory is supported by observations by Nielsen et al. (1996) as they observed the immune response to repeated rowing bouts. The neutrophils during the first trial followed the sequence observed in the ET, while in the subsequent bouts the neutrophil counts peaked during exercise and decreased in recovery.

A more detailed picture of the immune response can be observed by examining the relative changes in lymphocyte subpopulations determined by flow cytometric immunofluorescence. The percentage of $CD16^+$ (NK) and $CD8^+$ (T cytotoxic/suppressor) lymphocytes increased post-exercise, while $CD3^+$ (T), $CD4^+$ (T helper), $CD4^+$ 25^+ (activated T helper) lymphocytes and $CD4^+/CD8^+$ ratio decreased post exercise. All of the shifts in subpopulations of lymphocytes during the exercise treatment recovered to values not different than pre-test after 1 hr of recovery. Decreases in the $CD4^+/CD8^+$ ratio have been suggested to be indicative of immunosuppression, as it is a characteristic of diseased states, but the significance of observed changes in this parameter in response to exercise has also been questioned (Nieman et al., 1994). Similar changes in relative percentages of lymphocyte subpopulations have been well documented in literature following other short duration, high intensity exercise modes (Field et al., 1991; Hinton et al., 1997). The data is also very similar to that observed by Nielsen et al. (1996) with the exception that they observed no change in the relative concentration of $CD8^+$ lymphocytes throughout the rowing exercise test. It can be misleading to emphasize the relative decrease in a subpopulation as a larger increase in one subpopulation may lead to a decrease in another. The decrease in relative levels of some of the lymphocytes is often attributed to the

relatively large increase in $CD16^+$ cells (Nieman, 1997).

Other researchers have estimated the absolute counts of lymphocyte subpopulations by multiplying the relative concentrations determined by immunofluorescence times the lymphocyte counts to indicate cell trafficking (Pizza et al., 1995; Nielsen et al., 1996). The absolute concentrations of $CD3^+$ and $CD8^+$ were significantly elevated in response to the simulated rowing exercise in both trials post-test, but decreased to value not different than pre-test at recovery. The absolute concentrations of $CD4^+$ was also significantly elevated post-test, but decreased during recovery to a value significantly lower than pre-test. An indication of the activation state of T lymphocytes can be shown by the response of $CD4^+$ 25^+ cells (Pizza et al., 1995). The absolute concentration was significantly reduced at recovery compared to post-test values, while $CD8^+$ 25^+ did not change throughout the test in either trial. A decreases in the concentration of $CD4^+$ 25^+ cells indicates a reduction in the presence of the IL-2 receptor analog on the surface of the $CD4^+$ cells. This suggests a reduced sensitivity to activation as IL-2 is a known stimulator of lymphocyte proliferation. The largest increase in one subpopulation of cells was observed in $CD16^+$ lymphocytes, increasing 8 fold. This subpopulation did exhibit an main effect for trial as well as time as the RT elicited a significantly higher recruitment of $CD16^+$ cells than ET. Despite the very large increase in concentrations, the concentration of $CD16^+$ lymphocytes had returned to pre-test levels by the end of the one hour of recovery in both trials.

The significance of these changes in the subpopulations of lymphocytes, either in relative numbers or absolute concentrations is not clear. Although a decrease in T lymphocytes in recovery has been likened to having less soldiers on the battlefield (Nieman, 1997), others have been cautious to make the link between observations in the blood compartment and alterations at potential sites of infection (MacKinnon, 1992). Nielsen et al., (1996;1998) demonstrated similar increases in the subpopulations post-exercise, but they reported decreases in absolute concentrations of $CD3^+$ and $CD8^+$ during recovery. It is difficult to determine the significance of these observations as some differences in study methodology exist between the trials. This group performed their

immune analysis on isolated lymphocytes that were frozen and thawed before analysis, where as the present study used fresh whole blood for immunophenotypic analysis. Gradient isolation has been reported to alter the relative composition of some subpopulations (Field et al., 1991) and would contribute to these observed differences.

Changes in the immune cell populations and subpopulations show only a limited picture of the immune response. In such a complicated and coordinated system it is important to consider how the activities of these cells are influenced in response to the exercise treatment. Changes in the proliferative response of isolated lymphocytes in response to stimulation with a mitogen decreased in response to rowing exercise in both trials. This apparent suppression of lymphocyte activity has been reported previously (Field et al., 1991; Hinton et al., 1997; others). The change in activity however, is usually attributed to alterations in the relative concentration of the respondent subpopulation (as $CD16^{+}$ increases) and the decrease is not observed on a per cell basis (Fry et al., 1992; Shinkai et al, 1992). In the present study the 24 hr proliferative response to simulation with PMA decreased post-test but returned to a value not different than pre-test during the 1 hr recovery. The Con A stimulated 24 hr proliferation decreased from the post-test, but was still depressed after 1 hr of recovery. The Con A mitogen stimulates mainly T cells and since this change is not parallelled by changes in the subpopulations of lymphocytes it may be suggested that this suppression is present at the per cell basis. This is a unique observation as Nielsen et al. (1998) have only reported a change in post-test proliferation in response to the PHA mitogen in one study and this change was not present in the recovery sample.

The activity of neutrophils was investigated by testing the capacity of the cells to generate reactive oxygen species (oxidative burst) in response to stimulation. The activity of these phagocytic cells that form the first line of defence against invading pathogens have not been studied in the literature when rowing has been used as the exercise stimulus. During the assay the mean channel fluorescence increased sequentially with stimulation in the 5, 10 and 15 min samples indicating that some cells were responsive to stimulation. No difference was observed between the trials or during the testing session

for the 0 and 5 min time points. A significant decrease in mean channel fluorescence was detected at the 10 min and 15 min time point in the post-exercise sample compared to the pre-test and recovery values in both trials.

The significance of these observations is difficult to interpret as the multi-functional activities of neutrophils have been shown to change in different directions depending on the function studied (Smith, 1995). Some functions such as generation of reactive oxygen species (ROS) have increased following exercise of varying intensities and durations (Suzuki et al., 1996; Huupponen et al., 1995). Bury and Pirnay (1995) reported an increase in degranulation following longer duration exercise, while Robson et al., (1999) found a decrease in degranulation and oxidative burst following two different intensities and durations. Other researchers have found decreases in neutrophil functions such as generation of super oxide anions and microbicidal activity following exercise (Kettler et al., 1996; Hack et al., 1992). Overall, it is thought that higher intensity exhaustive exercise impairs most neutrophil functions with the exception of phagocytosis and degranulation (Rincon, 1994; Smith, 1997). Direct comparisons to these studies are difficult as several different methodologies and mitogens are used to assess the different functional activities of phagocytosis, oxidative burst and bacterial killing.

Whether these observations of changes in function are of clinical significance in terms of increasing resistance to infection is not clear. Some researchers are eager to propose mechanisms suggesting impaired function while others are cautious to suggest cause and effect relationships. Smith (1997) has been careful to point out that even with large shifts in activities, a critical line may need to be crossed before the observations become biologically significant and vulnerability to infection increases. Robson et al., (1999) has proposed that the large increase in neutrophil concentrations observed in peripheral blood may be indicators of potential for immune suppression. They suggest that repetition of several training sessions may diminish immune defence by depleting the immediately available pool of neutrophils from marrow thus increasing risk to infection. Others have suggested that a "refractory period" may exist post-exercise where neutrophils that are previously activated may be unresponsive to further activation and

create a window of opportunity during which opportunistic infections could take hold (Woods et al., 1999). The observations of decreases in function of neutrophils post-test and lymphocytes post-test and in recovery indicate an impairment of immune function but clinical significance is not clear. These observations are different than what has been observed previously in males, and when rowing following weight cutting is undertaken outside in a hot, humid environment potential for immune suppression is increased. Therefore this area warrants further study.

Conclusions

The results of the investigation demonstrate that a 2 hr rehydration period is sufficient to restore hydration to a level where performance is not impaired when 3.33% of body mass was lost by fluid restriction and sweating over the 24 hr prior in lightweight female rowers. Present findings also show that there was little difference in the immune response between the ET and RT in cell shifts and in function. However, the immune response to the simulated rowing exercise in general indicated that some impairment of immune response was present following one hour of recovery. Furthermore, the observation that the RT produced tympanic temperatures that were higher than ET suggests a higher level of heat stress in the RT. Thus, it is concluded that in lightweight female rowers 24 hr weight loss of 3.33 % does not impair performance or immune function. It is further concluded that the simulated rowing performance does compromise several immune parameters in lightweight female rowers, although the physiological significance is not known. The evidence that heat stress is greater in the RT should be considered in future studies as the could become a major concern when the exercise is performed outside in a hot, humid environment

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Table 3-1. Change in body mass of lightweight female rowers over the course of the dehydration/rehydration protocol (n=7).

Baseline (kg)	Dehydrated (kg)	Loss (kg)	Loss (%)	Rehydrated (kg)	Gain (kg)	Euhydrated (kg)
61.14±3.37	59.11±3.26[¥]	2.03±.15	3.33±.14	60.85±3.44	1.74±.36	60.99±3.49

[¥] Different than all other weigh-ins ($p<0.05$).

Table 3-2. Immune cell counts in response to the performance of a 2000m simulated rowing performance by lightweight female rowers in both a euhydrated and dehydrated/rehydrated state. Concentrations were determined by CBC and are reported as Means and standard deviations $x10^9$ cells/L (n=5).

Cell Type	RT1	RT2	RT3	RT4	ET1	ET2	ET3	ET4
WBC	5.68± .75	7.28± 1.65	13.16± 1.16¥	6.24± 1.56	4.24± .74	4.18± .74	8.64± 1.22*	7.16± 2.80*
Neutrophils	3.72± .75	5.08± 1.81	6.84± 1.79†	4.42± 1.62	2.10± .55	2.02± .58	3.04± .99	5.58± 2.71*
Lymphocyte	1.44± .17	1.74± 1.04	5.30± 1.90‡	1.36± .15	1.64± .35	1.66± .30	4.56± 1.03‡	1.10± .20
Monocytes	.34± .05	.30± .07	.76± .19‡	.30± .07	.34± .05	.32± .04	.78± .16‡	.44± .11

The following abbreviations are used in this and other tables for the sample time points: RT1 - hypohydrated weigh in; RT2 - pre-test (after 2hr rehydration period); RT3 - immediately post 2000m simulated rowing test; RT4 - following 1hr recovery period; ET1 - euhydrated weigh in; ET2 - euhydrated pre-test; ET3 - immediately post 2000m simulated rowing test; ET4 - following 1hr of recovery period. ¥ Different than all other values ($p<0.05$). * Different than weigh in (ET1) and pre-test (ET2) values ($p<0.05$). † Different than weigh in (ET1) pre-test (ET2)and post-test (ET3) values ($p<0.05$). ‡ Different than weigh in (RT1, ET1), pre-test (RT2, ET2) and recovery (RT4, ET4) values ($p<0.05$).

Table 3-3. Relative concentrations of lymphocyte subpopulations as determined by immunofluorescence using flow cytometry in response to a 2000m simulated rowing test in two different hydration states in lightweight female rowers (n=7).

Flourescent Label	RT2	RT3	RT4	ET2	ET3	ET4
$CD3^+$	77±12	58±12*	78±5	77±9	55±17*	73±17
$CD4^+$	44±8	27±7*	43±7	47±5	24±9*	40±7
$CD8^+$	30±4	39±5*	29±5	30±6	39±5*	27±4
$CD16^+$	10±4	33±3*	14±9	12±7	28±10*	11±5
$CD4^+$ 25^+	3±2	2±1*	3±2	2±1	1±1*	2±1
$CD4^+/CD8^+$	1.45±.27	.69±.15*	1.51±.30	1.63±.39	.64±.34*	1.54±.30

* Different than pre-test (RT2, ET2) and recovery (RT4, ET4) values ($p<0.05$).

$CD14^+$, $CD20^+$ and $CD8^+25^+$ were not detectable

Table 3-4. Estimated concentration of lymphocyte subpopulations in response to a 2000m simulated rowing test in two different hydration states in lightweight female rowers (n=5). The concentrations indicated are expressed as means and standard deviations $x10^7$ cells/L.

Flourescent Label	RT2	RT3	RT4	ET2	ET3	ET4
$CD3^+$	121±42	289±80*	105±13	132±26	254.±51.*	87.±21.
$CD4^+$	83±63	134±36*	57±14¥	79±20	110±21*	46±13¥
$CD8^+$	53±36	199±81*	41±9	48±11	174±51*	31±8
$CD16^+$	16±11	180±79*	22±12	17±4	124±58*	11±5
$CD4^+ 25^+$	5..99±6.65	7.06±4.55	2.91±2.16†	3.21±2.87	5.71±3.07	2.62±1.52†

* Different than pre-test (RT2, ET2) and recovery (RT4, ET4) values ($p<0.05$). ¥ Different than pre-test (RT2, ET2) and post-test (RT3, ET3) values ($p<0.05$). † Different than post-test (RT3, ET3) values ($p<0.05$). No interaction effects were observed. All indicated significance are main effects for time with the exception of the concentrations of $CD16^+$ where a main effect for trial was also noted. The RT overall had a significantly different response than the ET ($p<0.05$).

$CD14^+$, $CD20^+$ and $CD8^+25^+$ were not detectable

Table 3-5. Lymphocyte proliferative activity in response to a 2000m simulated rowing test in lightweight female rowers in two hydration states (n=5). Proliferation is represented by the absolute counts indicating the incorporation of ^{3}H thymidine in cell cultures following stimulation with PMA and Con A.

Mitogen	RT1	RT2	RT3	ET1	ET2	ET3
Unstim	601.2	1128.8	1023.2	626.8	3389.2	1975.6
	±426	±524	±500.9	±357.2	±3492.9	±1359.4
PMA	23362.8	22681.4	58952.8	19827.0	28863.4	32023.6
	±18136.6	±8075.7	±41556.2*	±6919.2	±16273.5	±18804.9*
Con A	41068.8	24810.2	58314.0	49212.4	39612.8	51948.2
	±12809.4	±7491.3	±31477.6	±13174.8	±27439.7	±23990.5

* Different than pre-test and post-test values ($p<0.05$), main effect for time only.

Table 3-6. Neutrophil oxidative burst activity in response to a 2000m simulated rowing test in lightweight female rowers in two hydration states (n=7). Oxidative burst is represented as mean channel fluorescence of neutrophils stimulated with PMA determined by flow cytometric analysis of the oxidation of dihydrorhodamine-123 to rhodamine-123.

Trial	O min	5min	10min	15min
ET pre	32±23	482±423*	1087±818*	1492±1062*
ET post	24±29	439±443*	897±826*¥	1260±1115*†
ET recov	27±27	577±477*	1190±868*	1679±1005*
RT pre	39±37	930±788*	1352±755*	1790±970*
RT post	23±26	590±386*	1140±748*¥	1514±857*†
RT recov	39±49	723±633*	1324±848*	1807±1028*

* Different than all other time points with each sample ($p<0.05$). ¥ Different than the 10 min time point of the pre-test and recovery sample ($p<0.05$). † Different than the 15 min time point of the recovery sample ($p<0.05$).

Table 3-7. Neutrophil oxidative burst activity in response to a 2000m simulated rowing test in lightweight female rowers in two hydration states (n=7). Oxidative burst is represented as stimulation index of the mean channel fluorescence of neutrophils stimulated with PMA determined by flow cytometric analysis of the oxidation of dihydrorhodamine-123 to rhodamine-123.

Trial	0-5 min	5-10min	10-15 min
ET pre	18.37±24.52*	1.07±0.85	0.38±0.21
ET post	29.55±33.52*	0.88±0.79	0.38±0.20
ET recov	25.27±26.78¥	0.92±0.65	7.68±19.45
RT pre	39.42±38.26*	0.68±0.49	0.32±0.15
RT post	36.39±34.22*	1.13±0.80	0.46±0.36
RT recov	26.85±31.06¥	2.46±4.0	0.47±0.37

*SI is significantly different than the 5-10 and 10-15 minute SI ($p<0.05$). ¥SI is significantly different than the 10-15 minute SI ($p<0.05$). All effects are main effect for time only with no effects for trial and no interaction. All effects are also within assay time points. There were no main effects for time or trial between trials.

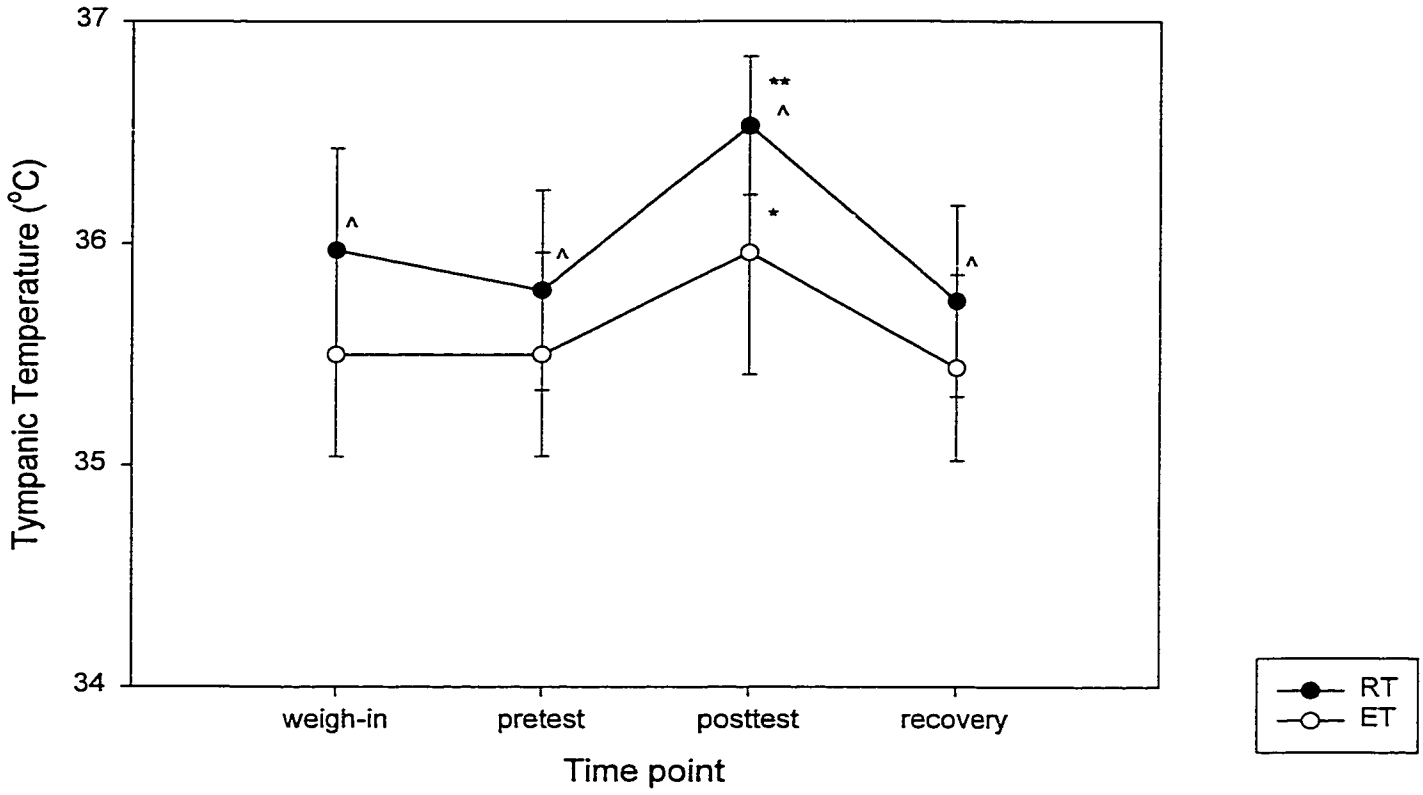

Fig. 3-1 Tympanic temperatures made throughout the 2000m simulated rowing test in a euhydrated and dehydrated/rehydrated state in lightweight female rowers (n=7).

^ - different than corresponding timepoint of ET ($p<0.05$)
* - different than all other measurements of ET ($p<0.05$)
** - different than all other measurements ($p<0.05$)

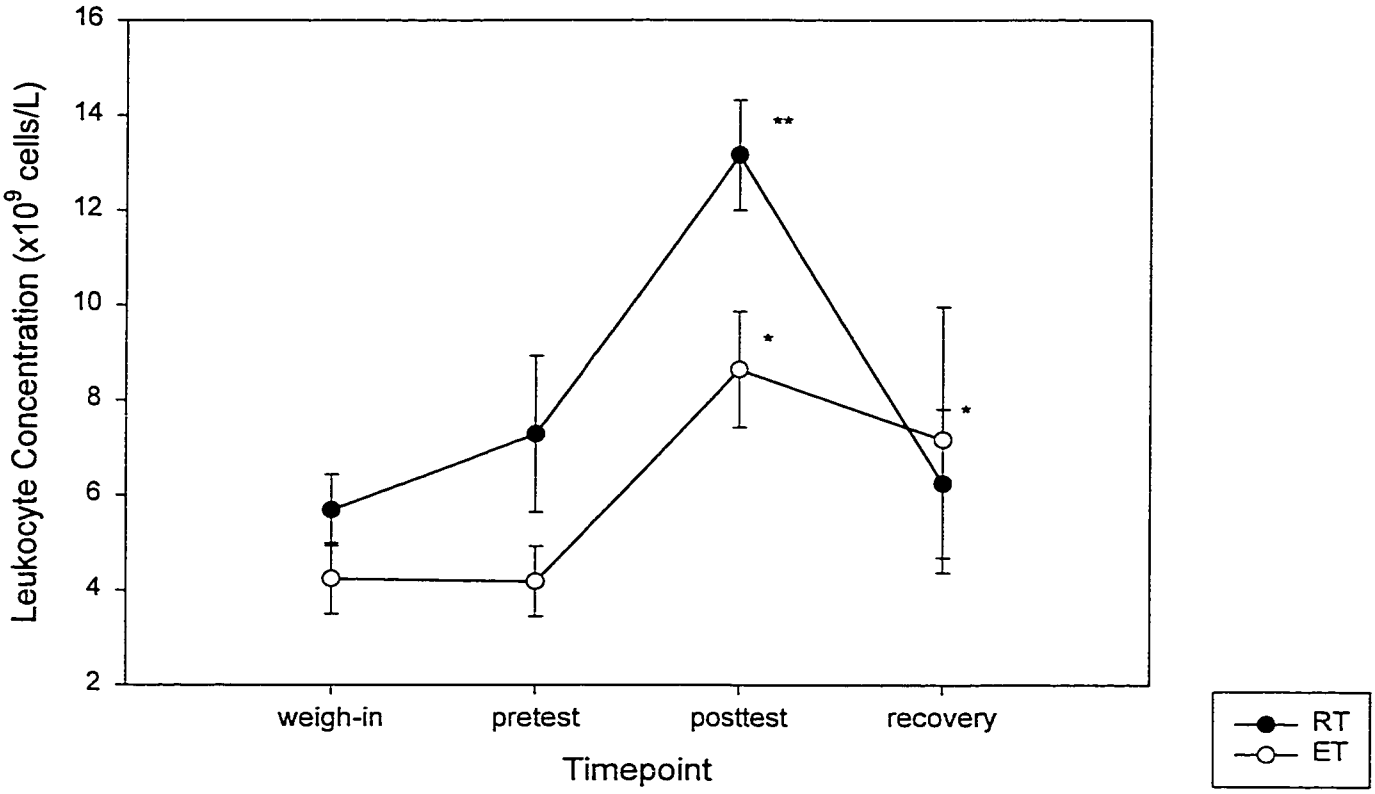

Fig. 3-2. Leukocyte counts made throughout the 2000m simulated rowing test in a euhydrated and dehydrated/rehydrated state in lightweight female rowers (n=5).

** - different than all other values ($p<0.05$)
* - different than the pre-test and weigh-in values for the ET ($p<0.05$)

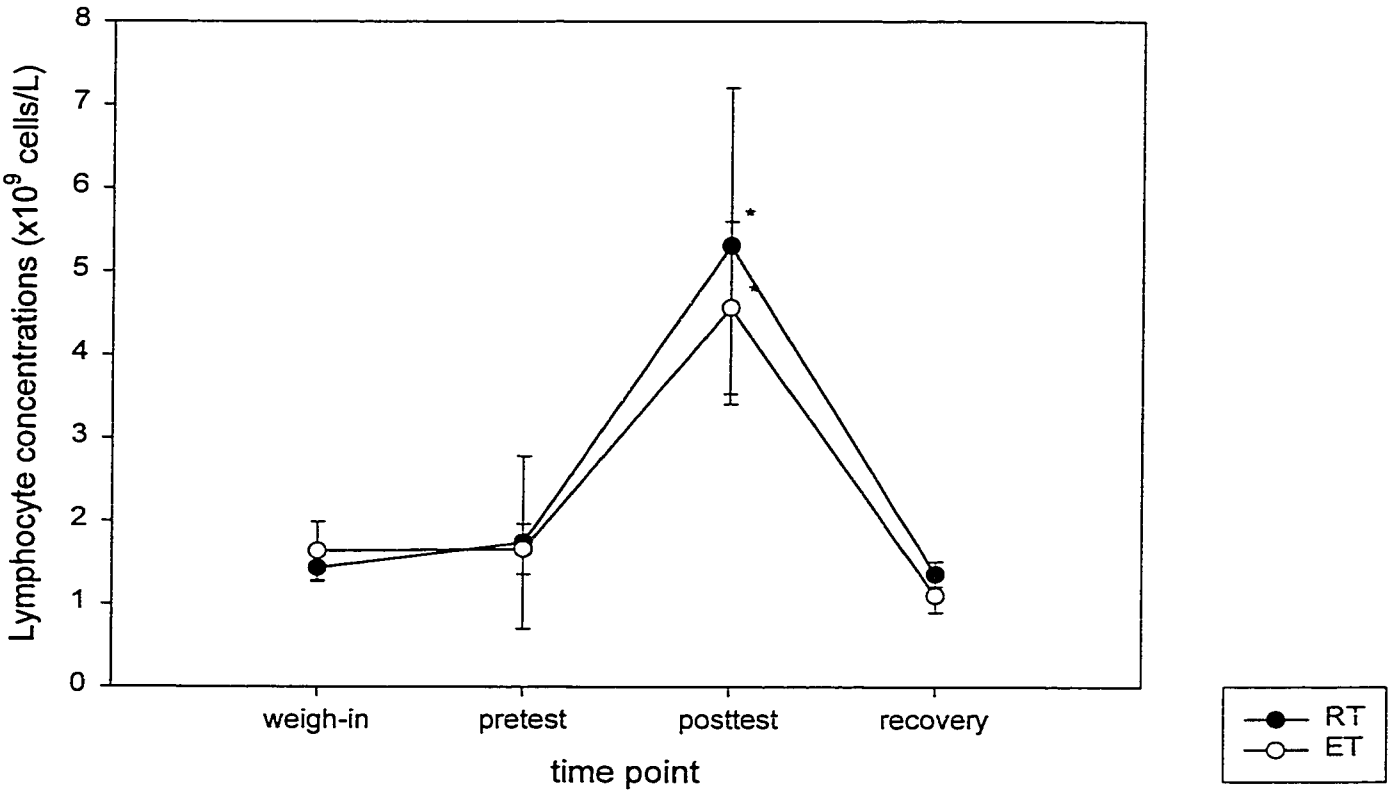

Fig. 3-3. Lymphocyte counts made throughout the 2000m simulated rowing test in a euhydrated and dehydrated/rehydrated state in lightweight female rowers (n=5).

* - different from weigh-in and pre-test and recovery values ($p<0.05$).

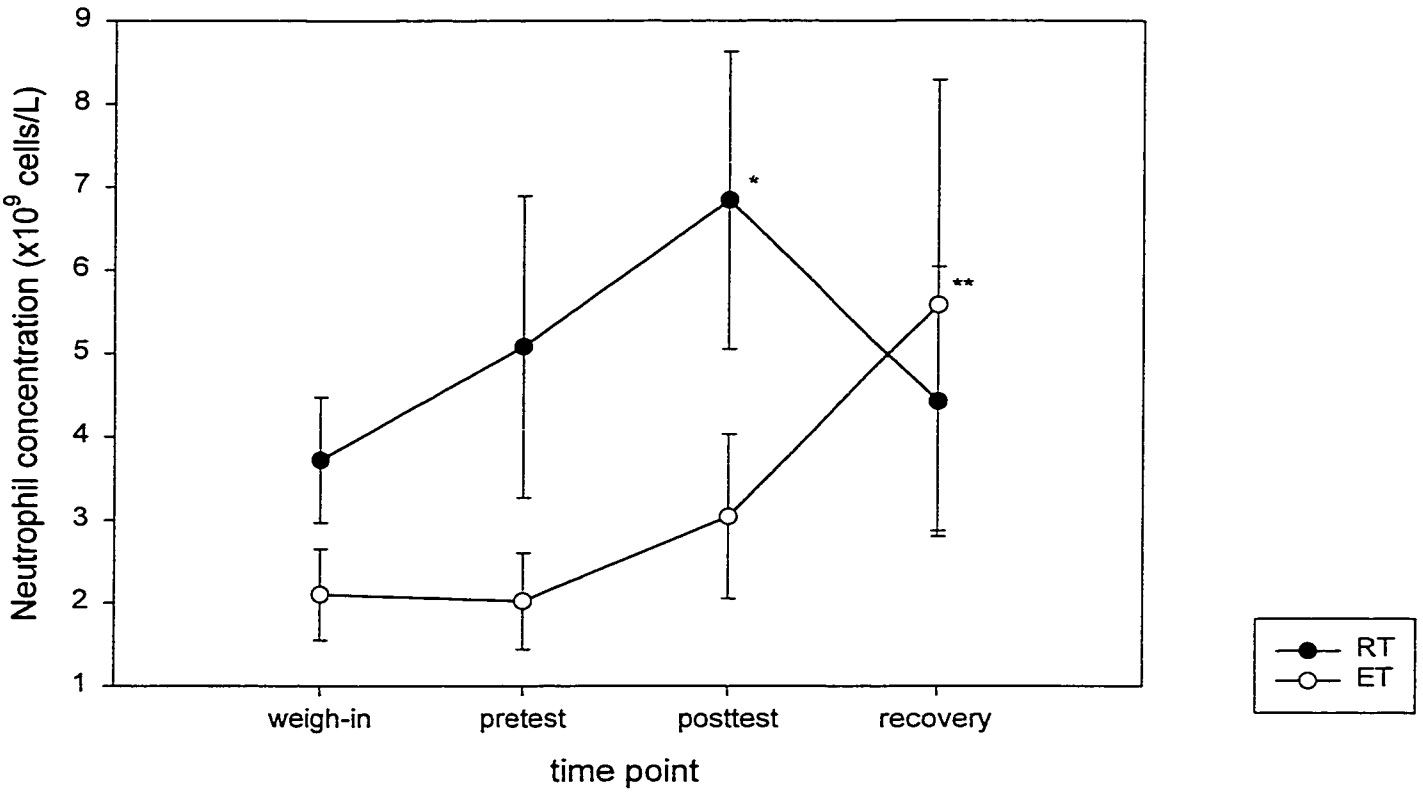

Fig. 3-4. Neutrophil counts made throughout the 2000m simulated rowing test in a euhydrated and dehydrated/euhydrated state in lightweight female rowers (n=5).

* - different than weigh-in, pre-test and post-test values of ET ($p<0.05$)
** - different than weigh-in and pre-test values of ET ($p<0.05$)

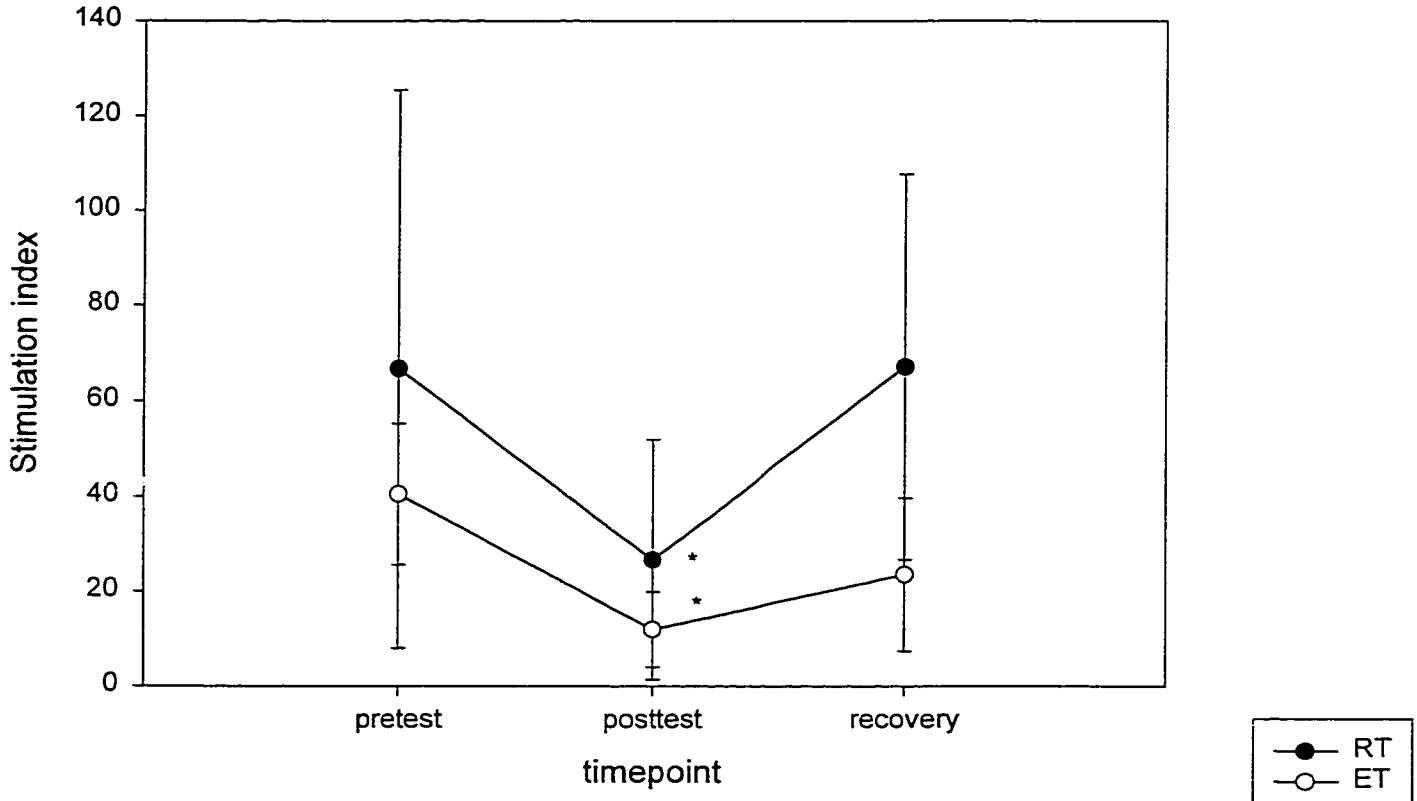

Fig. 3-5. Lymphocyte activity represented by stimulation index for lymphocyte proliferation in response to PMA stimulation during a euhydrated and dehydrated/rehydrated experimental trials in lightweight female rowers (n=5).

* - different than pre-test and recovery values ($p<0.05$)

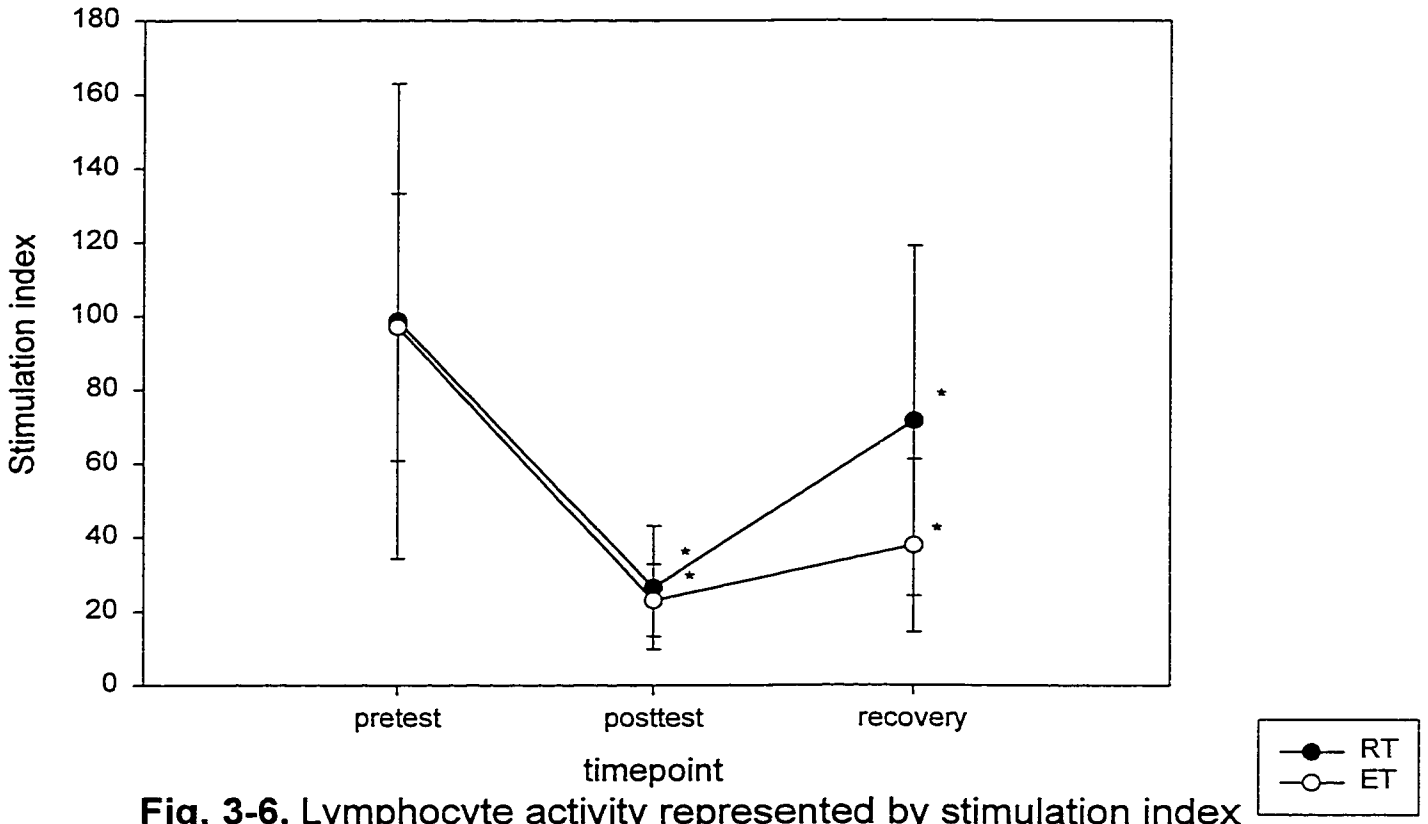

Fig. 3-6. Lymphocyte activity represented by stimulation index for lymphocyte proliferation in response to Con A stimulation during a euhydrated and dehydrated/euhydrated experimental trial in lightweight female rowers (n=5).

* - different than pre-test values ($p<0.05$)

CHAPTER 4
GENERAL DISCUSSION AND CONCLUSIONS

Many researchers have recently undertaken investigations in the exercise immunology field. Two main approaches have been taken in conducting this research: one, aiming to understand how exercise can positively affect the immune system; and two, aiming to maximize the benefits from time spent training and in so doing, minimize lost training days due to illness. In performing investigations to further understand these two broad areas, research has detailed the immune response to several different modes, durations and intensities of exercise.

This investigation had two main purposes; 1) to determine the impact of a simulated rowing race in a potentially hypohydrated state on the lightweight female rowers; and 2) to determine the effect of a short term weight restriction protocol using fluid restriction and hypohydration through sweating on performance and the thermoregulatory response in these subjects.

Hypotheses

The hypothesis for the two questions, respectively, are:
1). It was hypothesized that the immune response of the female rowers in the hypohydrated state would be different than the response in the euhydrated state. Furthermore, it was hypothesized that the response would also be different than that noted previously to rowing exercise in men.
2). It was hypothesized that the hypohydration protocol would lead to a decreased performance and impaired thermoregulatory response during the simulated rowing trial.

Experimental Design

1) Healthy female rowers were recruited as subjects and baseline body mass determined.
2) Subjects were required to lose 3.5% of their body weight through a combination of fluid restriction and light exercise over the 24 hr prior to the test (RT). In the ET no weight restriction practices were performed.
3) The order in which each subject was to perform the testing sessions were randomly assigned.
4) Following "weight cutting"(RT) or reporting to the lab (ET), subjects were required to complete a 2000m simulated rowing test on the Concept II rowing machine with serial blood samples taken for immune analysis.
5) Calculations of the main and interaction effects between trials and over time (weigh-in, pre-test, post-test and recovery), were made for all immunological and thermoregulatory measurements.

Based on the study design, several assumptions were made in order to form the conclusions of this investigation.
1) All subjects consumed a similar diet and fasted the morning of the test.
2) All subjects abstained from exercise in the 24 hr prior to both of the testing sessions.
3) All subjects utilized only light exercise indoor and fluid restriction to reach the assigned weight loss.
4) All subjects performed the two 2000m rowing trials with maximum physical and mental efforts.
5) External stresses and differences in cyclic hormonal levels did not contribute to intra-subject variability.

General Discussion

This study, unlike a similar study done on the performance effect of short term weight loss in rowing using men as subjects (Burge et al., 1993), showed no impairment of performance due to the hypohydration protocol. It is important to note however, that several design modifications were used in the present study compared to the design used by Burge et al., (1993). These changes were made in order to make the sequence of events that were undertaken by the subjects in order to lose weight similar to that undertaken by rowers at competitions. The weight loss period was moved from the evening prior to the morning of the rowing test, and the percentage of body mass lost was reduced to a level equal to the maximum weight than is commonly lost by these athletes. The time between the two treatments was also reduced as the week given in the previous study was judged to be to long when attempting to control for variations in timing of the female subjects menstrual cycle. These modifications attributed to the lack of a detectable performance impairment. When considering the 72 hr recovery period between rowing tests, it is possible that this was inadequate when the hypohydrated trial was performed first. Three subjects performed the RT first, while four performed it second. Of the three who performed the RT first, two had difficulty completing the ET trial three days later. One had to be verbally encouraged to finish after almost stopping and the other did stop requiring a second test to be performed two days later. These subjects reported feeling weakened at the start of this trial. This might have been an example of simply two poor tests not related to the exercise treatment, but one can look to the trend that all five other subjects did see an increase in performance time on the RT compared to ET to suggest that some performance effect may not have been detected by this study.

Furthermore, this study aimed to investigate whether the immune response to rowing exercise was affected by the 24 hr weight cutting protocol, and if the immune response in female subjects was different than what had been previously observed in men. Answers to these questions could provide a more clear understanding of why rowers have one of the highest incidences of illness among several athlete groups (Castell et al., 1996). With the

exception of differences in the shifts in concentration of some immune cell populations in response, the immune response was similar between the two trials. For the most part the cell trafficking responses were similar to what has been reported in the literature on rowing (Nielsen et al., 1996;1998) and other high intensity activities (Field et al., 1991; Hinton et al., 1997). Leukocytosis consisting of lymphocytosis and neutrophilia have commonly been reported following intense exercise bouts. Most of the shifts in the subpopulations of lymphocytes occurring post-test returned to normal values after 1 hr of recovery. An exception was the absolute concentration of $CD4^+$ (T helper) lymphocytes, and those T helper cells expressing the activation marker $CD25^+$ which were suppressed below the pre-test level following 1 hr of recovery, suggesting a decrease in the activation state of this subpopulation. Observations such as these have lead to the proposal of the "Open window hypothesis" to suggest that an impairment of immune function may occur in the recovery period following exercise, opening the window for infection to take hold (Pedersen & Ullum, 1994). This observation has not been made in any of the previous studies on rowing and the immune system in men, in fact Nielsen et al., (1996) observed a decrease in the $CD8^+$ (T suppressor) subpopulation of cells in response to rowing exercise following 1 hr of recovery. A further potential indicator of an impaired immune response is the decrease in lymphocyte proliferation as measured by the stimulation index of a 48 hr proliferation assay when stimulated with Con A. The decrease in proliferation observed immediately post-test has been observed in several studies, but is often attributed to relative shifts in the subpopulations of cells and not a change in the function of lymphocytes on a per cell basis (Hinton et al, 1997;). The large increase in NK cells leads to less T lymphocytes in the assay immediately following exercise. The unique observation in this study is that the proliferation of lymphocytes was suppressed following the 1 hr recovery period when the relative proportions of all immune cell subpopulations had returned to values not different from pre-test values. This depression of lymphocyte function has not been observed following shorter term exercise such as rowing previously, but it has been observed following the longer duration exhaustive exercise and suggested as clinical evidence of immmunosuppression.

Often when studies are performed on acute exercise bouts, the question of what effect chronic administration of the exercise stress would have on the immune system should be a concern for future studies. This is also the case with rowing exercise. Future studies must look at the chronic effects of rowing training and repeated competitions over a season to attempt to understand why this population of athletes suffers incidences of illness that are as high as any other population of athletes despite the short duration of the exercise stress (Castell et al., 1996). The observation in the present study that the RT lead to an increased level of heat stress compared to the ET despite no difference in performance should be considered to be of major importance to future studies. This has consequences for potential for heat illness, performance, immune function, and study design concerning the menstrual status of female subjects if the exercise bout is performed in a hot, humid environment similar to what they would encounter in the summer racing season.

Higher temperatures and humidity may impair the ability of the rehydration period to fully replace the fluid lost. The heat and humidity can increase fluid loss through sweating, decrease evaporative cooling and thus lower the threshold of body mass reduction where maximum aerobic power and performance is impaired (Sawka & Pandolf, 1990). In addition to potential performance impairment, the elevation in core temperature becomes a major concern where hypohydration could lead to heat injury (Sawka & Pandolf, 1990). Furthermore, the increase in heat stress can have an effect of adding to the already high level of stresses affecting these athletes and have more threatening consequences for the immune response. The combination of environmental and exercise stress have been reported to have at least an additive effect. Therefore, the combination of two levels of stress that had not been immunosuppressive previously, could readily impair immune function if the challenges occur simultaneously (Shephard, 1999; Brenner et al., 1998; Pedersen et al., 1994).

In the present study we did not control for phase of the menstrual cycle, but did document the phase of the subjects. We found no evidence that the reported phase of the subjects effected the response of the immune system to the exercise stress which is in

agreement with previous research (Northern et al, 1994; Zelazowska et al., 1997). However, the research in this area is limited and the phase of menstrual cycle could influence results of future studies combining environmental heat stress and rowing exercise following weight cutting. Evidence that tolerance to heat stress is lower in the mid luteal phase compared to early follicular phase in non oral contraceptive users (Tenaglia et al., 1999) may have consequences on performance and immune function if phase is not controlled for.

This study provides evidence that the exercise stress induced by a simulated 2000m rowing test may have negative consequences for immune function. Athletes and researchers should pay particular attention to the observations that the immune response of these female subjects is somewhat different than the response of male subjects. Athletes should also consider any means necessary to avoid potential for impaired performance or increased risk of illness which may lead to lost training days. Hopefully this study will lead to others that can address the much greater risk that higher ambient temperatures and humidity can bring to weight loss practices associated with rowing performances in male and female subjects.

Limitations of the Study

1) The performance of the rowing simulation in the controlled environment of the laboratory does not completely represent the potential for heat stress and heat injury when the weight cutting and exercise bout is performed outside in a hot humid environment such as is the case in many of the summer regattas in which rowers compete.

2) This study is performed using an acute exercise bout. The results could be considerably different when this type of activity is performed and repeated several times over a rowing season.

3) This study attempted only to investigate dehydration induced weight cutting. In reality

athletes will often combine moderate to severe caloric restriction when attempting to cut weight and may also turn to diuretics for maximum weight loss.

4) We attempted to, but could not completely control for the female menstrual cycle and its potential influence on the immune systems response to the treatment. Two of the seven subjects were oral contraceptive users and it was not possible to test the other subjects on the same cycle day. Attempts were made to keep the testing days as close as possible so that the athletes were tested in the same phase but in some cases this was not possible.

5) Due to the limited number of available lightweight female rowers the study had a small number of subjects (n=7). This was further complicated in a few of the assays, as problems with one or more of the sample analyses eliminated the entire subject from the ANOVA analysis limiting the useful subject n to 5. These were specifically the lymphocyte proliferation assay and the CBC determination which also affected the absolute lymphocyte subpopulation calculation.

6) In future testing I would cover the Concept II performance monitor only allowing the distance and stroke rate to be shown so that the testing would be more similar to the performance on the water. This would prevent the athletes from focussing completely on the split times to row close to what they did on the previous test.

7) The time between testing of 72 hours did not seem to provide adequate recovery for some of the rowers. Feelings of fatigue from the previous testing session were reported when the hypohydrated test was performed first. This may have influenced the performance on the second test and masked a performance effect. The time between testing was kept small to try to prevent cyclic hormonal changes from causing differences between the two testing sessions, but in the future perhaps more recovery time between the tests (ie.1 week as in Burge et al.,1993) would make the performance differences more clear.

Conclusions

The present investigation has provided evidence that indicates that an high intensity simulated rowing exercise can lead to significant changes in the immune response of lightweight female rowers as indicated by lymphocyte proliferation and neutrophil oxidative burst. The combination of this exercise stress with a short term dehydration and rehydration protocol did not impair performance, but did result in an elevation of body temperature compared to the exercise stress alone. Therefore, an all-out 2000m simulated rowing test can alter immune response in lightweight female rowers. However, a 3.33% decrease in body weight via fluid restriction and sweating over 24 hr followed by a 2 hr rehydration period did not impair performance, but did increase heat stress in a controlled lab environment.

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Appendix A

DEMOGRAPHIC QUESTIONAIRE

Name:_____ **Date:**_____

Address:_____ **Telephone:**_____(H)

_____ _____(W)

_____ **E-mail:**_____

Date of Birth:_____ **Age:**_____

Answer the following questions as accurately as possible.
Please ask for clarification where needed.

MENSTRUAL CYCLE

1. At what age did you have your first menstrual period? _____(years)

2. Have you taken oral contraceptive pills within the last 6 months? Yes / No

3. Are you currently taking oral contraceptive pills? Yes / No

4. Is your menstrual cycle regular (ie. every 24-35 days)? Yes / No

☞ if "NO" (answer the next four questions).
(a) When was the last time you menstruated? _____
(b) How many periods do you usually have in a year?_____

(c) On average, how many days does your period last?_____
(d) What is the longest time you have gone without a period?_____

☞ if "YES" (answer the next four questions).
(a) How many periods do you usually have in a year?_____
(b) On average, how many days does your period last?_____
(c) What is the interval of days between your period? Indicate the number of days between "Day 1" (onset of flow) of a period and Day1 of the next period. _____
(d) When was the last time you menstruated? _____

If known indicate the last three "Day 1's" of your menstrual cycle:

_____

_____

_____

Rowing History

1. How long have you been rowing?_____

2. How many times have you cut weight to row?_____

3. Do you plan to cut weight to row in the future?_____

4. How much weight have you lost to make weight? Max_____ Ave____

5. How have tried to loose weight in the past? (ie. dieting, dehydration etc.) _____
_____
_____
_____

Appendix B

FACULTY OF PHYSICAL EDUCATION AND RECREATION
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PHONE: 780-492-2018 FAX: 780-492-2364

The Physiological and Performance Effects of Simulated Race Rowing on Female Lightweight Rowers in a Euhydrated and Hypohydrated State

Investigators:
Michael Penkman, B.Sc. 433-9838
Gordon J. Bell, Ph.D. 492-2018
Vicki Harber, Ph.D. 492-1023
Catherine Field, Ph. D. 492-2597

INFORMED CONSENT FOR EXERCISE TESTING AND TRAINING

I,_____(please print your name) agree to participate in a research project conducted by the above named investigators studying the nature of the physiological and performance effects of a simulated race rowing test in two different hydration states (normal and low hydration). I agree to participate in the exercise testing procedures to the best of my ability. I understand that I may withdraw from the study at any time, for any reason without any consequence. I also understand that the staff conducting the test will discontinue any procedures if any indications of abnormal responses become apparent. I understand that prior to performing any test listed below I will have the opportunity to question and discuss the exact procedures to be followed.

Physiological Testing:

1. Anthropometry - measurement of height, weight and age will be recorded.

2. Ventilation Threshold (VT) and VO_2max - a continuous 12 minute exercise test of increasing intensity to exhaustion on a rowing machine while monitoring metabolic responses and heart rate.

3. Two 2000 m Rowing Tests on different days - metabolic responses, power output, time and heart rate will be measured during a simulated rowing race of 2000 m.

4. Serial blood samples (7 ml each) from an arm vein will be obtained on three different days. Four samples will be taken on each of the testing days from an indwelling catheter on the back of the forearm.

Testing Protocol:

Two testing sessions will be required, both tests (regular hydration and low hydration) will be performed in the morning (9 am) and separated by 72 hours. Testing will occur following a 24 hour dehydration protocol for the dehydrated test. The dehydration protocol will require the

subjects to reduce their body weight by 3.5% of their body weight by fluid restriction and light exercise. This will be monitored by a weigh-in 24 hours prior to and the morning of the testing session. Testing will require 3 separate visits. The pre-test blood will require about 15 minutes. The total time required for physiological testing will require approximately 10 hours in total for the project.

Risks:

The exercise tests will require maximal physical and mental effort. However, the effort required will not be greater than that experienced during rowing performance. The tests represent little risk to healthy, active individuals involved in sport. The dehydration regimen will require the subjects to reduce body weight through fluid restriction and light exercise, and this may result in an increase in core body temperature. Also, as the projects seeks to determine any possible changes in immune function due to this practice, there is potential for the immune system to transiently suppressed. These risks are justified in that the weight loss is not extreme, and within the normal competitive situation these athletes practice (Burge et. al, 1993). The study also hopes to promote safer weight making practices with its results. The blood samples will be performed by an individual trained in the venipuncture technique. There is a risk of infection, bruising and small hematoma at the site if not properly cared for. The care for all these procedures will be explained to all subjects.

Burge, C.M., Carey, M.F., & Payne, W.R. (1993). Rowing performance, fluid balance, and metabolic function following dehydration and rehydration. Medicine and Science in Sports and Exercise, 25 (12), 1358-1364.

Consent

I acknowledge that I have read this form and I understand the test procedures to be performed and the inherent risks and benefits involved from participation in this project. I consent to participate understanding that I may withdraw at any time without consequence. I may expect a copy of this consent form at the outset of the study and a report of my personal results at the end of the study. I understand that the data collected will be used in a research publication and will remain in possession of the investigator to ensure confidentiality. I also understand that I may make any enquiries concerning any procedure that I do not completely understand. I consent to participate in this research project.

Name:_____ Signature:_____
(print) (sign)

Address:_____ Date: _____

_____ Phone:_____

Witness:_____ Investigator:_____

Signature:_____ Signature:_____