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Effects of Liquid Carbohydrate Feeding on Salivary IgA During Exercise in a Heated Environment

Director: Dr. Brent Ruby

ABSTRACT

PURPOSE: The purpose of this study is to quantify the effects of prolonged exercise and carbohydrate (CHO) feedings in a heated environment on the salivary immune marker IgA. **METHODS:** In a randomized double-blind design, 11 recreationally active males and females walked for 120 min at ventilatory threshold (VT) in the heat (39°C) on two occasions while fed CHO or sweet placebo (Pla). Females performed both heat trials within 7 days post-menses to minimize hormonal variations in metabolism. CHO was delivered in a 7% solution and based on fat-free mass, $1.16 \text{ g} \cdot \text{kg FFM}^{-1} \cdot 60 \text{ min}^{-1}$ ($0.7 \text{ g} \cdot \text{kg body mass}^{-1} \cdot 60 \text{ min}^{-1}$). Blood samples were collected at baseline, at 30 min intervals during exercise, and immediately post-exercise. Timed saliva samples were collected at baseline, post-exercise, and 1 and 6 hrs post-exercise. **RESULTS:** VT resulted in a highly homogenous physiological response ($P=0.70$). Plasma glucose levels became significant only at 120 min of exercise ($P<0.01$). Saliva flow rate had no significant variations. Salivary IgA (s-IgA) concentration and s-IgA secretion rate had significant declines when compared to rest in the CHO and Pla trials ($P<0.035$), however, there were no significant variations across trials. **CONCLUSION:** These findings suggest that heat exacerbates the effect exercise has on s-IgA parameters and hot ambient temperatures delay any benefit CHO supplementation may have on attenuating a diminished immune system in moderate temperatures. This project was funded through a grant provided by the Missoula Technical and Development Center.

**Effects of Liquid Carbohydrate Feeding on Salivary IgA
During Exercise in a Heated Environment**

By

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B.A. Concordia College, Moorhead, MN, 1991

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for the degree of

Master of Science

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Chapter One: Introduction

Introduction

There is substantial anecdotal information from athletes, trainers and coaches, suggesting that rigorous physical activity increases the risk of upper respiratory tract infection (URTI) ^{24, 45, 48}. The Wildland Firefighter, to some, an unorthodox endurance athlete, becomes a unique example of this paradigm with similar physiological battles.

Wildland firefighting demands a high energy output averaging 7.5 kcal per minute, over 400 kcal per hour, and up to 6000 kcal per day ⁵¹. Rigorous activity and heavy exertion (physiological stress) increases circulating epinephrine and cortisol, which may suggest alterations to the normal immune response ^{40, 46}. Coupling this high-energy work with psychological stress, the physical environment, long hours and multiple consecutive shifts, little sleep, the difficulty in maintaining energy and/or hydration balance achieves a dangerous scenario, which may result in acute and/or chronic overtraining and immunosuppression.

The immune system is comprised of an intricate system of organs, tissues, cells and molecules, which try to maintain homeostasis within all living beings. All forms of stress from psychological to physical influence this attempt at homeostasis. Once a pathogen has accessed the body the integrated immune response is initiated. This study is most interested in the mucosal immune system and with the increased risk for upper respiratory tract infections.

The mucosal immune system is a network of immune structures at mucosal surfaces throughout the body that provides effective protection at distal sites from the original site of antigen presentation ⁵. The mucosal immune system is systemic involving many aspects of the body, however, the focus of this investigation includes the respiratory tract and the salivary IgA marker.

Effective immunity in the URTI challenge begins at the mucosal surfaces with s-IgA playing a major role in defense of homeostasis. This specific response in association with a general immune response is required to maintain an asymptomatic state to URTI's. A state of immunosuppression weakens this system and it is suggested that levels of s-IgA may influence the risk for URTI.^{20, 31, 35, 54}

The immune system is a high-energy system when battling pathogens. Most immune responses involve cell replication and the production of proteins with specific functions. The mechanisms by which nutrient deficiencies affect the immune system have been classified as either direct or indirect. A nutritional deficiency is said to have a direct effect when the nutritional factor being considered has primary activity within the lymphoid system and an indirect effect when the primary activity affects all cellular material, or another organ system, which acts as an immune regulator¹⁰. For example, carbohydrate availability directly affects a number of leukocyte functions but also exerts an indirect effect on the lymphoid system through its influence on circulating levels of the catecholamines, adrenocorticotrophic hormone and cortisol¹⁷ during exercise. In addition, carbohydrate supplementation modifies the immune response imposed by exercise^{18,25}. In what way does it "modify" the immune response

Wildland firefighters attempt homeostasis despite numerous stresses including physical and psychological which have all been shown to depress the immune system including the specific marker, s-IgA^{11, 20, 31, 35, 54}. One of the many stressors affecting the battle for homeostatic maintenance for Wildland Firefighters is heat. These athletes are working in the hottest days of the year and fully dressed in fire protective clothing while maintaining a high level of energy output suppressing fires, up to 6000 kcals/day⁵¹ in harsh environments.

Ambient temperatures exceeding body temperature stress thermoregulatory systems in the body to release excess heat, which is only exacerbated with exercise.

Housh et al.²⁶ showed that acute exposure to various temperatures, up to 34°C, have no effect on s-IgA levels. Wildland Firefighters are exposed to high ambient temperatures exceeding 34°C for extended periods of time. Performance, measured as either time to exhaustion or work output during a specific task, is reduced in the heat^{12, 14} and proportional to the increase in core temperature.

Adequate carbohydrate intake is an essential component to heavy training schedules, with a current recommended intake of 8-10g of carbohydrate per kg body mass for athletes training more than 2 hours per day²³. The primary concerns addressed with such recommendations are restoration of muscle and liver glycogen. Additionally, glucose becomes important to cells of the immune system as an immediate source of energy in the battle against pathogens, thus, adding to the importance of adequate energy balance and carbohydrate intake.

If carbohydrate supplementation attenuates immunosuppressive episodes (decreased s-IgA) then emphasizing proper carbohydrate intake and energy balance may help protect the Wildland Firefighter from acute and/or chronic overtraining leading to immunosuppression and ultimately an URTI.

Purpose

The purpose of this study was to determine the effects of prolonged exercise in a heated environment on changes in salivary IgA. This study examined and compared levels of s-IgA and other general immune markers during exercise in the heat with two hydration/feeding regimens (carbohydrate and Pla).

Research Hypothesis

Hypothesis One

Carbohydrate feedings will attenuate a decrease in immune markers (s-IgA and CBC's) after prolonged exercise in a heated environment.

Hypothesis Two

Plasma glucose and lactate will be elevated during the carbohydrate trial.

Justification

Collectively, the literature suggests a depressed immune function after rigorous physical activity, which has a cumulative effect. Many studies have found depressed immune markers, s-IgA ^{20, 31, 35, 54}, and suggest this as the cause for the prevalence of URTI's in endurance athletes. However, only one study has (Housh et al., 1991) looked at the effects of heat and exercise on s-IgA and no study has determined the result of prolonged exercise in a heated environment on the immune system, specifically s-IgA.

Significance of Study

In immunology and exercise studies, there has been only one published study ²⁶ that has used temperature to examine s-IgA levels after exercise. This study will be the first to investigate the effects of excessive heat and prolonged exercise on the immune system.

Most previous immunology and exercise studies have selected arbitrary set percentages of VO_{2max} . There is an evolving argument centered upon relative, ventilatory and lactate thresholds, and absolute, VO_{2max} , intensities. Relative intensities are derived from metabolic responses of an individual's physiology, which are a direct reflection of fitness level. Whereas absolute intensities account for the maximum volume of oxygen an individual may transport in a given short-term effort, which in large part is genetic (i.e. lung volume). Lung volume is much less variable due to fitness level than is the accumulation of lactate in the blood. Among the many physiological responses, ventilatory threshold is an indirect response to the accumulation of lactate in the blood.

Varying lactate thresholds may have significant effects on exercise metabolism, even in participants with a similar VO_{2max} ⁷. An illustration of this would be if two

participants had the same VO_{2max} but different lactate thresholds, 55% and 75% of their VO_{2max} . If the protocol calls for an intensity of 65% of VO_{2max} , one participant would be exercising at a higher intensity than the other with a heavier reliance on carbohydrate. Ultimately, this is a great difference when a study's results are looking at comparative factors and metabolic influences.

In a repeated measures design the reduction of within group variability is vital in order to obtain a more homogenous response to variables introduced. Reduced variability between participants improves the power of detecting differences. The use of relative intensities to control for exercise intensity is used to address these methodological concerns of metabolic response studies.

Rationale for the Study

The investigation of heat and carbohydrate supplementation on the immune marker, s-IgA, is necessary to more fully understand immunosuppressive episodes due to acute bouts of work. Understanding how a replication of the stressors challenging the Wildland Firefighter may give additional insight into nutritional and/or hydration issues in order to maintain proper health over the course of a fire season.

Limitations

- i/ Instrumentation. Inherent error is associated with all instrumentation. However, using trained testers, calibrated equipment and accounting for potential drift will minimize error.
- ii/ Non-randomized sample. The sample will not be randomly selected. Random selection is used to equalize compared groups on extraneous variables. This repeated measures design will use the same participants for both trials (carbohydrate vs. Pla) therefore eliminating confounding variables (e.g. immunity levels, fitness levels, psychological thresholds, training).
- iii/ Hydration and nutritional status. Hydration occurred at regular intervals and was not limited. Nutrition, carbohydrate supplementation, was given at regular intervals during

the carbohydrate trial. Depending on the circumstances, both hydration and nutrition is more limited for the Wildland Firefighter. Many times they are limited to what they carry in their backpack.

iv/ Stress level. Replication of actual environmental and psychological stressors of the Wildland Firefighter in the field are attempted and unfortunately minimized due to the fact that they cannot be duplicated.

v/ Isolated exercise task. Work of the Wildland Firefighter encompasses whole body movements and exercise. This study's exercise task will only replicate one aspect of their working environment, walking/hiking, in attempt to provide sufficient physiological stress.

vi/ Duration. The length of exercise, although prolonged for an exercise study, is quite short in the reality of Wildland Firefighter work.

vii/ Load carriage. Participants will carry no extraneous load beyond their own body weight. The Wildland Firefighter always carries a pack, which varies in weight.

Delimitations

i/ Participant type. The results of this study may have implications for Wildland Firefighters, therefore, minimum fitness qualifications as posed on the Wildland Firefighters are required for the study participants. These include a recreationally active lifestyle and a minimum of $50 \text{ ml} \cdot \text{kgFFM}^{-1} \cdot \text{min}^{-1}$.

ii/ Hormonal limitations. Hormones influence metabolism, therefore, female participants had an additional qualification of no external hormonal influence, which predominantly is obtained through exogenous birth control measures. This investigation will also only accept eumenorrheic females, since hormonal influence is critical to metabolism, helping in the determination of early follicular presentation. Additionally, the female participants will be tested in the early follicular phase to capture their hormone cycle (progesterone and estrogen) at their least influential point.

- iii/ Environment. The actual working environment of the Wildland Firefighter is highly variable, whereas, the study will maintain a controlled environment (39°C).
- iv/ Intensity. This investigation will attempt to sufficiently stress the participants, as would a portion of the Wildland Firefighters typical workload.
- v/ Hydration and nutritional status. Water was readily available and not limited. During the carbohydrate trial, a carbohydrate solution was provided regularly and uniformly based on each participant's fat free mass.

Definition of Terms

Early Follicular Phase. Days 1-7 immediately after menses stops during the female hormonal milieu.

Ventilatory Threshold. An intensity of exercise at which metabolic gases are affected and determined in the following ways:

ventilatory equivalents. The first rise in the ventilatory equivalent of oxygen without a concurrent rise in the ventilatory equivalents of carbon dioxide.

excess CO₂ production. An increase from steady state to an excess production of CO₂.

modified V-slope method. Plots the minute production of carbon dioxide over the minute utilization of oxygen.

Hydrostatic weighing. Determination of fat-free mass (FFM) using net underwater weight while taking into consideration the individual's residual volume.

Residual Volume (RV). The volume of air remaining in the lungs following a maximal expiration.

50 ml·kgFFM⁻¹·min⁻¹. A minimum standard of which all participants must meet in order to establish a minimum fitness level, in order to resemble Forest Service standards.

Rested. No high intensity exertion in previous 24 hours and no exercise in previous 12 hours.

Fasted. No caloric intake for at least 10 hours.

50-25-25 Diet. A dietary intake for the three days prior to heat stress trials consisting of 50% carbohydrates and the balance made up of protein and fat in an attempt to mimic a typical firefighter's diet out on the fire line.

Fluid Intake. Carbohydrate/Plasma feedings, which is variable, based on a carbohydrate delivery of a 7% solution per kg FFM. Water *ad libitum* for initial trial (A) and duplication of that volume for second trial (B) for repeated measures statistical analysis.

Carbohydrate Feeding. Supplied in a solution according to FFM, ensuring equal intake for all participants based on metabolically actives, not simply body weight.

Exercise Intensity. 100% Ventilatory Threshold.

Heated Environment. The heat chamber will maintain a constant temperature of 39°C ($\pm 1^{\circ}\text{C}$) for each heat stress trial.

Chapter Two: Review of Literature

Research design of salivary Immunoglobulin A and exercise

Results vary from studies investigating the effects exercise has on s-IgA levels ¹⁶. Methodological differences between previous studies include variations in intensity, duration, saliva sample collecting protocol, expression of s-IgA concentrations and environmental (including controlling for participant state of being). All of the previous s-IgA studies reviewed established exercise intensities at set percentages of VO_{2max} . Saliva sample collection has been received from whole mixed saliva ^{20, 34, 35} or specific glands, e.g. parotid, ^{31, 54} with this collection being stimulated ^{32, 53} or unstimulated ^{3, 21, 54}. The fitness levels of sample populations have ranged from recreational/sedentary/variable fitness level ^{3, 21, 34, 54} to elite fitness level ^{20, 33, 54}.

Incidence of Upper Respiratory Tract Infections

Several epidemiological studies ^{24, 28, 43-45, 48, 49} have reported the effects of exercise on the risk of developing upper respiratory tract infections, emphasizing endurance training and endurance events such as marathons and ultra-endurance competitions. Peters and Bateman ⁴⁸, Nieman, Nehlsen-Cannarella et al. ⁴⁶, Heath et al. ²⁴, among others, found an increased incidence of URTI's associated with higher intensity and/or longer duration endurance events and training. Nieman, Johanssen et al. ⁴⁵ assigned mildly obese females to walking or nonexercising groups. During this time, the exercising group had improved fitness, and increased natural killer cells with a lower incidence of URTI compared to their sedentary controls. This investigation concluded that intensity of exercise plays a major role in immunosuppressive vs. immuno-enhancing activities.

Salivary IgA and exercise

A state of immunosuppression weakens the immune system. Due to the nature of s-IgA's role in the mucosal immune system it is suggested that levels of s-IgA influence the battle against URTI's ^{20, 31, 35, 54}. All of which investigated the effects of exercise on s-

IgA resulting in decreased levels of s-IgA. However, the subjects fitness level ,saliva collection protocols and intensities have varied among previous research. Studies have also demonstrated no change in s-IgA levels ^{34, 35} after sub-maximal exercise, also leading to the conclusion that intensity/duration (amount of physiological stress) plays a predominant role in depressed levels of s-IgA. It is generally accepted that higher intensities promote immunosuppressive episodes while lower intensities result in more individual responses.

Carbohydrate supplementation and the immune system (s-IgA)

Studies ^{19, 41, 42} have shown that carbohydrate ingestion at regular intervals during strenuous exercise attenuates immunosuppression based on immune system markers. To our knowledge only one study ¹ has demonstrated the attenuation of s-IgA depression with carbohydrate supplementation.

Chapter Three: METHODOLOGY

Setting

All physiological testing took place in the Human Performance Laboratory, McGill Hall #121, The University of Montana.

Participants

Twelve recreationally active males (n=6) and females (n=5) served as participants.

Participants were healthy and free of prescribed medication. Prior to participation, each participant provided consent as approved by the University Internal Review Board.

Preliminary Testing and Data Collection

Preliminary testing included an informative session where testing methods were explained to each participant. Baseline measures of peak oxygen consumption (peak VO_2) and body composition were obtained prior to the extended exercise sessions in the heat.

Peak VO_2 treadmill test

Peak oxygen uptake was measured using a graded maximal treadmill test (Quinton Q65 treadmill) and open-circuit spirometry (Modified ParvoMedics system with an LB-2 CO₂ Gas Analyzer; OM-11 O₂ Analyzer, and heated Hans-Rudolph pneumotach). Criteria for peak VO₂ was established with matching two of three criteria: base levels of 1) RER (1.1) 2)% of estimated maximal heart rate (min. 90-95% maximal HR) 3) Decreased rate of O₂ consumption (plateau). Prior to each test, the metabolic system was calibrated using a three-liter calibration syringe (Spirometrics, Inc.) and medical gases of known concentration (5.17% CO₂; 15.2% O₂; Balance N₂). Metabolic data was recorded in fifteen-second intervals. From the baseline data, a Ventilatory Threshold (VT) was established for each participant using the technique of Gaskill et al.¹⁵. Exercise intensity for the extended exercise trials was established as the treadmill speed and grade associated with 100% of the calculated speed and grade at the ventilatory threshold. Female participants performed all testing at the end of menses (preliminary testing) and during the early follicular phase (Trials A and B).

Body Composition

Body composition was assessed using hydrostatic weighing with a digital scale (Exertech, Dresbach, MN) at estimated residual volume³⁰. Net underwater weight was calculated from 100 sampling intervals over a 1.5 second recording period. Multiple underwater trials were repeated to establish no less than three measures within 100 grams and an average taken.

Testing Protocol

Participants recorded food intake for 3 days prior to each test session, choosing foods to maintain a dietary intake of 50% carbohydrates with the balance consisting of protein and fat. Nutrient intake was assessed using a food processor program for Windows by ESHA Research (Salem, OR). Participants were required to track nutrient and water

intake, as well as activity, during the 5 hrs between the 1 hr and 6 hr sample times of trial A and subsequently duplicate these variables for trial B.

Exercise Testing

For each test session the participants reported to the Human Performance Laboratory at 7:00 a.m. in a minimum 10-hour fasted and rested condition. Participants were required to have refrained from intensive exercise for twenty-four hours prior to the testing and have avoided all exercise for 12-15 hours before testing. The participants were also free of symptoms related to infection, specifically upper respiratory infections. Immediately after arrival, participants were encouraged to void their bladder prior to obtaining a nude body weight (Befour B1 digital scale, model # PS6600T). Participants inserted core temperature probe (YSI Precision 4000 A Thermometer) to a depth of 10 cm, which was kept in place for temperature recording throughout the extended heat-stressed exercise bout. An intravenous teflon catheter (18 or 20 gauge) was placed in an antecubital vein and kept patent using a continuous saline drip (.45 normal saline). After a 3-5 minute rest period upon IV insertion, a baseline blood sample was obtained.

Prior to treadmill test administration each participant produced a saliva sample in a four minute (-4 – 0 minutes), unstimulated collection procedure via expectoration. Samples were stored at -80°C until subsequent analysis. Following the initial sample collections, trial A's extended exercise sessions were initiated at an intensity equal in grade and treadmill belt speed to 100% of the baseline VT. Each extended exercise session included 120 minutes of treadmill walking in an environmental heat chamber (39°C). Modifications to intensity, when absolutely necessary, were made in trial A and documented so as to ensure completion of 120 minutes heat-stressed exercise bout and subsequent duplication in trial B.

Additional saliva samples were obtained at immediately post-exercise, 1 hr post-exercise and 6hr post-exercise sample times(-4-0, 120-124 minutes, 60-64 post testing and 6hr post testing). Following the test initiation, blood sampling, heart rate (HR) monitoring, ratings of perceived exertion (RPE) according to Borg et al.⁴, core temperatures and carbohydrate or Pla feedings occurred at 30-minute intervals (0, 30, 60, 90, 120 minutes). HR values determined using Polar, chest-strap HR monitors. Carbohydrate was supplied according to fat free mass (FFM) to give CHO by $\text{g} \cdot \text{kg FFM}^{-1} \cdot 60 \text{ min}^{-1}$ based on $0.7 \text{ g} \cdot \text{kg body mass}^{-1} \cdot 60 \text{ min}^{-1}$ or $1.16 \text{ g} \cdot \text{kg FFM}^{-1} \cdot 60 \text{ min}^{-1}$ (Mitchell et al, 1989) delivery ensuring equal intake for males and females according to energy requiring tissues. CHO and Pla delivery followed a double blind protocol. All trials were conducted at the same time of day to control for any diurnal effects on s-IgA levels²⁷.

Expired air samples were obtained for gas analysis in 5-minute intervals every 30 minutes (0-5, 25-30, 55-60, 85-90, 115-120 minutes) using the same metabolic system and procedure discussed above. Dehydration⁹ was controlled for with fluid intake within the 7% CHO solution at 30-minute intervals and water *ad libitum* during trial A and subsequent water intake duplication during trial B. Fluid intake was measured by tracking CHO solution ingested, water intake and saline infused during testing. Fluid output was measured via urine collection during exercise trial and prior to measure of nude body weight post exercise, including volumes collected at 60 (if needed) and 120 minutes of each test.

Blood Sampling

During each trial, a total of six blood samples were obtained (0, 30, 60, 90, 120 minutes; 5-8 ml/draw). Samples were obtained from an antecubital vein with participants supine for baseline and post exercise samples, while the sample collection during testing was obtained with participants straddling treadmill belt for minimal delay of exercise. Four ml of the 0, 60, and 120-minute blood draws were placed into a sterile Vacutainer tube

containing K₃ EDTA. These samples were set-aside at room temperature until subsequent analyses immediately post exercise (complete blood count – CBC, Western Montana Clinic). CBC analyses were completed within 4 hours post collection. At 30, 60, 90, and 120 minutes 500 µl of the remaining blood drawn will be immediately transferred to a tube, mixed with 1000µl 7% perchloric acid and spun down. Samples were stored at –30°C until subsequent analysis. The perchloric acid extract was used for the glucose, glycerol and lactate assays. Glucose, glycerol and lactate concentrations were assayed using a Spectronic 401 spectrophotometer (Milton Roy) and were analyzed using an enzymatic spectrophotometric technique (glucose Sigma assay procedure #16-UV; glycerol Sigma assay procedure #337). Lactate was analyzed according to Lowry (1976). All metabolite values were corrected for plasma volume change per individual participant.

Saliva Sampling

Each trial consisted of four, four-minute saliva collections (-4-0, 120-124 minutes, 60-64 minutes post, and 6hr post exercise). Unstimulated whole saliva was collected by expectoration into 15-ml plastic, sterilized tubes for the four-minute collection period. The sample was sealed and stored at –80°C until analysis. Saliva weight was measured to the nearest .01 g, and saliva total protein was quantified using the Coomassie® protein assay reagent, a modification of the Bradford Coomassie® dye binding colorimetric method. Salivary IgA was measured by enzyme linked immunosorbent assay according to the procedures of the Hunter Immunology Unit (royal Newcastle Hospital, Newcastle, NSW, Australia). The data was expressed as concentration of sIgA (µg ml⁻¹), concentration of sIgA relative to total protein concentration (µg min⁻¹), and sIgA secretion rate (µg min⁻¹). The saliva flow rate (µl min⁻¹) was calculated from measurement of the saliva divided by the collection time. Salivary density was assumed

to be 1.00g ml⁻¹. Saliva IgA secretion rate was calculated by multiplying saliva flow rate by saliva IgA concentration.

Statistical Analysis

Data are expressed as mean $\pm$ SEM. A student's t-test was used to analyze actual vs. expected %VT across trials. Salivary IgA measurements were analyzed using a 2-way repeated measures ANOVA (2 X 4, trial X time). A prior planned comparisons on the trial X time interaction including comparisons between trials at each time point and comparisons from pre to each time point within each trial. Experiment-wise alpha was adjusted to minimize type I error because the desired number of comparisons exceeded $k-1$ ($k-1=7$; $(0.05 \times 7)/10=0.035$). Lactate, glucose, HR and core temperature values were analyzed using a 2-way repeated measures ANOVA (2 X 5, trial X time). A prior planned comparisons on the trial X time interaction including comparisons between trials at each time point and comparisons from pre to each time point within each trial. Experiment-wise alpha was adjusted to minimize type I error because the desired number of comparisons exceeded $k-1$ ($k-1=9$; $(0.05 \times 9)/13=0.0346$). CBC values were analyzed using a 2-way repeated measures ANOVA (2 X 3, trial X time). A prior planned comparisons on the trial X time interaction including comparisons between trials at each time point and comparisons from pre to each time point within each trial. Experiment-wise alpha was adjusted to minimize type I error because the desired number of comparisons exceeded $k-1$ ($k-1=5$; $(0.05 \times 5)/7=0.037$).

Chapter 4: RESULTS

Subject Characteristics

All of the participants (n=11) of this study were recreationally active and currently involved in regular exercise. All female participants (n=5) performed both exercise trials in the early follicular phase of their menstrual cycle (within the first seven days post menses). Descriptive data are shown in Table 1.

Expression of exercise intensity during the heat stress exercise trial

The average exercise intensity was not significantly different for the two trials (99.4% and 97.75% of $\text{VO}_{2\text{VT}}$ for the Pla and CHO trials, respectively, see Table 2). There were no significant differences in the average values for oxygen consumption, exercise intensity, RER, sweat rate, percent change in plasma volume or change in body weight across trials (see Table 2). Values for HR, CT and RPE are presented in Table 3. Although there were no significant differences between the trials, each trial showed a significant increase in HR, CT and RPE at each time point compared to the initial 5-minute measures (Table 3).

Metabolites

Metabolite analyses are presented for only 10 participants as a result of sampling difficulty with one male subject. Multiple comparison analysis revealed significant differences in plasma glucose levels during the CHO trial at 30, 60, 90, and 120 minutes when compared to baseline (Figure 1). However, plasma glucose levels during the Pla trial revealed a significant difference from baseline at the 30-minute sample time only. Multiple comparison analysis further revealed a significantly higher plasma glucose during the CHO trial at 120 minutes in comparison to the Pla trial. (Figure 1).

There was a significant increase in plasma lactate levels during the CHO and Pla trials at 30, 60, 90, and 120 minutes compared to baseline (Table 4). Plasma lactate was significantly lower during the CHO trial at 60, 90, and 120 minutes compared to the Pla trial (Table 4). Similarly, there was a significant increase in plasma glycerol levels during the CHO and Pla trials at 30, 60, 90, and 120 minutes compared to baseline (Table 4). However, there were no significant differences between Pla and CHO trials (Table 4).

Blood immune markers

All blood immune marker analyses (CBC analysis) were performed using 10 subjects due to sample clotting, which occurred in one male's sample. Multiple comparison analysis revealed a significant increase in WBC, Lymphocytes, Neutrophils, and Monocytes during both the CHO and Pla trials at the post-exercise sample time when compared to baseline, while Eosinophils showed no significant difference over time (Table 5). There were no significant differences between CHO and Pla for any of the blood immune markers at any time point (Table 5). All values were corrected for any change in plasma volume.

Salivary immune markers

All salivary IgA analyses were done without two participants (n=9) due to viscous saliva and a missed sample. Multiple comparison analysis revealed no significant change in salivary flow rate ($\text{ml}\cdot\text{min}^{-1}$) compared to rest or across trials (Figure 2). A significant decrease in the concentration of s-IgA ($\mu\text{g}\cdot\text{ml}^{-1}$) was seen during the CHO trial at 1 hr and 6 hr post exercise compared to baseline. However, the Pla trial demonstrated a significant decrease in s-IgA ($\mu\text{g}\cdot\text{ml}^{-1}$) at only 6 hr post exercise (Figure 3). Figure 3 also shows that there were no differences across trials for s-IgA concentration ($\mu\text{g}\cdot\text{ml}^{-1}$).

Similar to s-IgA concentration ($\mu\text{g}\cdot\text{ml}^{-1}$), multiple comparison analysis revealed a significant decrease in IgA secretion rate ($\mu\text{g}\cdot\text{min}^{-1}$) during the CHO and Pla trials (pre vs. 6 hr post) (Figure 4). A significant decrease in IgA secretion rate ($\mu\text{g}\cdot\text{min}^{-1}$) was also noted during the CHO trial at 1 hr post exercise compared to baseline. However, there were no significant differences between the CHO and Pla trials.

This present data revealed a significant but transient increase in salivary protein concentration ($\text{mg}\cdot\text{ml}^{-1}$) at the post sample times for both the CHO (baseline= $2.69\text{ mg} \pm 0.44$ vs. post-exercise= $5.34\text{ mg} \pm 0.92$) and Pla (baseline= $2.82\text{ mg} \pm 0.52$ vs. post-

exercise=5.02 mg $\pm$ 0.62) trials. However, there were no significant differences across the trials for protein concentration.

Figure 5 shows the change in protein secretion rate for each trial. There was a significant decrease in protein secretion rate (mg min⁻¹) at the immediate post, 1 hr and 6 hr post sample times during the CHO trial. However, during the Pla trial, there was a significant decrease in protein secretion rate (mg min⁻¹) at the 6 hr post sample time only. The CHO trial also demonstrated significantly lower protein secretion rates (mg min⁻¹) at the immediate post and 1 hr post sample times in comparison with the Pla trial.

Figure 6 demonstrates a significantly lower IgA to protein ratio (μ g IgA:mg protein⁻¹) during the CHO trial at the immediate post, 1 hr and 6 hr post exercise sample times in comparison to baseline values. The Pla trial also demonstrated a significant decrease in this ratio (μ g IgA:mg protein⁻¹) for the immediate post and 6 hr post exercise sample times in comparison to baseline (Figure 6). There were no significant differences between the CHO and Pla trials.

Chapter 4: DISCUSSION

The primary finding of the current study indicates that liquid CHO supplementation does not attenuate the ensuing alterations of immunity in response to an acute (2hr) bout of exercise in the heat. In the current study, the combination of heat exposure, exercise intensity and duration resulted in maximal fatigue and altered immune markers (CBC and salivary data). Although previous research has evaluated the s-IgA marker in response to extended exercise bouts (1 to 2 hr) and/or competitive endurance events ^{1, 29, 34, 53, 54}, only one study has evaluated the s-IgA marker in response to short term exercise (30 minutes) in the heat ²⁶. In addition, to our knowledge only two investigations have incorporated liquid CHO supplementation during exercise in hopes of attenuating the s-IgA response ^{1, 2}. However, the effectiveness of liquid CHO

supplementation on the s-IgA marker during extended exercise in the heat has not been evaluated.

According to a recent review by Gleeson ¹⁶, the disparity in the literature for the exercise-induced changes in s-IgA is partly due to inconsistent exercise protocols and non-standardized data representation. In the current study, the exercise intensity was normalized to each subjects VT in an attempt to minimize variations the physiological response across subjects (Table 2). In addition, the physiological responses at VT are more closely related to training status and exercise performance compared to an arbitrary percentage of VO_{2PEAK} ⁸.

In the current study, plasma glucose concentration was similar between trials until 120 min. This suggests an impaired rate of gastric emptying in response to the high ambient temperature of 39°C or an increase in hepatic glucose production in response to the heat regardless of CHO ingestion. In agreement with the present study, Neufer et al. ³⁸ reported that gastric emptying is markedly reduced in individuals performing treadmill exercise in a hot (49°C) as compared with neutral (18°C) environment. Furthermore, high ambient temperatures may inhibit gastric emptying by reducing splanchnic blood flow ⁵⁰ and or gastric motility ⁵⁵. Previous research has demonstrated that exercise intensity can influence gastric emptying ¹³. Exercise performed at or below 70% VO_{2PEAK} does not apparently inhibit gastric emptying in comparison to the delay noted during exercise above this intensity. In the current study, subjects averaged 57% and 56% of VO_{2PEAK} during the Pla and CHO trials, respectively. Therefore, the apparent delay in gastric emptying noted in the present investigation cannot be fully explained by the intensity of exercise.

Our 7% glucose solution ³⁶ and volume ⁵² fits within the suggested ranges known to allow adequate gastric emptying and fluid delivery, at room temperature (22°C). In comparison, one previous study has suggested that a 10% CHO solution consumed during exercise in a 35°C environment (65% VO₂ peak, 120 minutes of treadmill running) did not compromise gastric emptying ⁴⁷. The type of CHO within the solution in combination with the heat may act to inhibit gastric emptying ³⁷, despite the beneficial effects of a high volume. These studies have suggested that glucose solutions inhibited gastric emptying more dramatically in comparison to fructose solutions when presented in equimolar solutions. Although the conditions are favorable for unimpeded gastric emptying in lower ambient temperatures, the current study is suggesting that hot environments may slow gastric emptying and thus absorption, regardless of intake volume, exercise intensity, or solution concentration

Hargreaves et al.²² has demonstrated that hepatic glucose production is increased during the last 30-min of a 40-min cycle ergometer exercise bout in a 40°C environment. Interestingly, there were no differences in the rate of glucose disappearance in response to the heat stress trial. Collectively, these data support the delayed response in plasma glucose noted in the present investigation.

In the current study, the salivary flow rate did not change in response to exercise. However, s-IgA concentration was suppressed for both post-exercise sample times (1hr and 6hr) during the CHO trials and for the 6 hr post-exercise sample time during the Pla trials, with no significant differences between trials. Although there was a trend towards an increase in the 6 hr post salivary flow rate (ml·min⁻¹, above baseline), the values were not statistically significant. A concomitant significant decrease in s-IgA concentration (µg·ml⁻¹) resulted in a significant decrease in the IgA secretion rate at the 6 hr post sample time during both the CHO and Pla trials. These results are in contrast to a

similar CHO feeding study ¹ in which mean IgA secretion rates (ml min^{-1}) demonstrated a slight but non-significant increase. These differences may be due to the possible contribution of a heat-induced inhibition of gastric emptying or simply an effect of the 40°C ambient temperature.

Interestingly, the combination of a possible heat-induced inhibition of gastric emptying and a heat-induced increase in fluid loss theoretically mimics the study of Bishop et al.¹. Bishop has suggested that the responses of salivary IgA are mostly affected by changes in fluid balance. Bishop et al.¹ has further suggested that the consumed fluid volume has a more dramatic influence on the s-IgA and flow rate than the CHO content of the consumed fluid. Expounding on this statement, these findings may indicate that fluid balance and core temperature as a reflection of ambient temperature are more critical at influencing the s-IgA marker. Nielsen ³⁹ suggested that an elevated core temperature is the ultimate cause of fatigue due to heat stress and that this may be the major contributor to altered immune states. Therefore, the combination in the present investigation may further exacerbate the process of compromised immunity. However, the process of CHO or Pla feeding along with ad libitum water intake resulted in minimal changes in plasma volume and body mass.

The current study revealed significant decreases in s-IgA concentration ($\mu\text{g ml}^{-1}$) during both the CHO and Pla trials compared to rest without finding a significant change between the CHO and Pla trials. This significant decrease in s-IgA concentration is in contrast to the increase noted by Bishop et al.¹. In addition, our results indicate significant decreases in IgA secretion rate (Figure 4) during both the CHO and Pla trials, demonstrating further alteration of the immune marker. The contrast with Bishop et al. indicate an acute, yet exacerbated immune response to prolonged exercise in the heat, which is not attenuated with the addition of liquid carbohydrate.

Correcting s-IgA concentration for total protein concentration may be misleading when the concurrent alteration in protein secretion rate does not parallel the alteration in s-IgA secretion rate. In addition, there is evidence stemming from past research indicating that protein secretion, predominantly amylase⁵⁶, increases during prolonged exercise³. Interestingly, as can be seen in Figure 5, protein secretion in the present study decreased significantly in both trials and is the only saliva related variable, which revealed a difference across trials. In the current study, the CHO trial demonstrated a decrease in protein secretion ($\text{mg} \cdot \text{min}^{-1}$) at the post exercise time point and throughout recovery. In contrast, the Pla trial demonstrated significantly lower values at the 6 hr post sample time in comparison to baseline values. Additionally, protein secretion rate was significantly lower at the 1 hr post sample time during the CHO trial compared to the Pla trial. As previously discussed, this interaction may be due to an exaggerated physiological response caused by a compounding effect of supplemental CHO during exercise at a high ambient temperature.

Interestingly, there were no significant changes the salivary flow rate in our study. This is similar to the data of Bishop et al.¹. Physical stress causes a stimulation of the sympathetic nervous system and a subsequent decrease in blood flow to the gut, leaving a majority of the blood flow to respond to the needs of the working muscles. The blood flow restriction includes the salivary glands, leading to decreased salivary secretion during exercise⁶. Despite the hot ambient temperature (likely causing an increase in skin blood flow), there were no significant changes in salivary flow rate over time during the CHO or Pla trials. This may in part be explained by the high ad libitum water intake, which, maintained close to normal hydration. Although this study did not reveal a significant change in saliva flow rate, the CHO trial did reveal a trend of attenuating any decrease in flow compared to the Pla trials. Therefore, the proposed inhibition of gastric

emptying may minimize the suggested benefits of supplemental liquid CHO to salivary flow¹.

Overall, the results of the current study maintain that CHO feedings (provided at an average rate of $1.16 \text{ g} \cdot \text{kg FFM}^{-1} \cdot \text{hr}^{-1}$ in a 7% solution) have no effect on attenuating acute alterations in immune function (based on changes in blood and salivary markers) during or after extended exercise in the heat. These results further demonstrate that the benefits of supplemental nutrition may be best achieved outside of the exercise bout in hot environments when gastric emptying is impaired. Further research should determine the effects of post-exercise CHO and nutrient intake on the restoration of immune function.

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Table 1. Physical characteristics of males (n=6) and females (n=5). Expressed as mean $\pm$ SD.

Age (years)	25.8 $\pm$ 8.1
Height (cm)	172.4 $\pm$ 10.4
Weight (kg)	73.9 $\pm$ 14.4
Fat Free Mass (kg)	62.4 $\pm$ 13.9
Peak Heart Rate (b min ⁻¹)	188 $\pm$ 6.5
Heart Rate at VT (b min ⁻¹)	149 $\pm$ 22.2
VO _{2PEAK} (ml kg ⁻¹ ·min)	59.5 $\pm$ 11.4
VO _{2PEAK} (ml kg FFM ⁻¹ ·min)	70.8 $\pm$ 10.1
VO ₂ at VT (ml kg ⁻¹ ·min)	35.4 $\pm$ 8.7
VT as % of VO _{2PEAK}	59.3 $\pm$ 8.8

Table 2. Actual workload intensities, sweat rate, plasma volume and body weight for the sub-maximal carbohydrate and placebo exercise trials. Expressed as mean $\pm$ SEM.

	<u>Placebo</u>	<u>Carbohydrate</u>
VO ₂ * (ml kg ⁻¹ .min ⁻¹)	34.8 $\pm$ 2.5	34.4 $\pm$ 2.3
%VO _{2VT} *	98.9 $\pm$ 3.7	97.5 $\pm$ 3.4
%VO _{2PEAK} *	57.0 $\pm$ 1.2	56.3 $\pm$ 1.2%
RER*	0.86 $\pm$ 0.01	0.89 $\pm$ 0.01
Sweat rate (g.m ²⁽⁻¹⁾ .min ⁻¹)	22.72 $\pm$ 1.78	22.58 $\pm$ 1.56
Plasma Volume (%)	-1.37 $\pm$ 1.44	0.08 $\pm$ 0.42
Change in Body Mass (kg)	-0.41 $\pm$ 0.25	-0.46 $\pm$ 0.29
Change in Body Mass (kg) [†]	-1.53 $\pm$ 0.40	-0.97 $\pm$ 0.37

*Averages calculated from steady state gas samples collected at 60 and 120 minutes.

[†]Corrected for fluid intake and output between pre and post nude body weights.

Table 3. Changes in HR, core temperature and RPE during the carbohydrate and placebo exercise trials. Expressed as mean $\pm$ SEM.

Time (min)	0	30	<u>Placebo</u> 60	90	120
HR (b $\cdot$ min ⁻¹)	158 $\pm$ 5.6	166 $\pm$ 6.5* [†]	171 $\pm$ 5.4* [†]	167 $\pm$ 4.8*	172 $\pm$ 4.9*
Core T (°C)	37.28 $\pm$ 0.10	37.86 $\pm$ 0.40* [†]	38.60 $\pm$ 0.39*	38.68 $\pm$ 0.41*	38.74 $\pm$ 0.46*
RPE	12.0 $\pm$ 0.65	14.3 $\pm$ 0.40*	15.4 $\pm$ 0.65*	15.4 $\pm$ 0.60*	16.6 $\pm$ 0.62*
	<u>Carbohydrate</u>				
HR (b $\cdot$ min ⁻¹)	157 $\pm$ 5.7	167 $\pm$ 5.8*	174 $\pm$ 4.8*	169 $\pm$ 5.4*	170 $\pm$ 5.2*
Core T (°C)	37.34 $\pm$ 0.07	38.34 $\pm$ 0.11*	38.98 $\pm$ 0.17*	38.89 $\pm$ 0.22*	38.98 $\pm$ 0.26*
RPE	12.2 $\pm$ 0.49	14.2 $\pm$ 0.33*	14.8 $\pm$ 0.53*	15.0 $\pm$ 0.60*	15.9 $\pm$ 0.77*

* p<0.0346 (experiment wise alpha <0.05) vs. rest.

† p<0.0346 (experiment wise alpha <0.05) vs. placebo.

Table 4. Changes in Lactate and Glycerol during the carbohydrate and placebo exercise trials.
Expressed as mean $\pm$ SEM.

	<u>Placebo</u>				
	rest	30	60	90	120
Lactate (mM)	0.79 $\pm$ 0.10	1.30 $\pm$ 0.24*	1.20 $\pm$ 0.19*	1.08 $\pm$ 0.13*	1.10 $\pm$ 0.11*
Glycerol (mM)	0.04 $\pm$ 0.007	0.06 $\pm$ 0.006*	0.06 $\pm$ 0.008*	0.07 $\pm$ 0.006*	0.11 $\pm$ 0.012*
	<u>Carbohydrate</u>				
	rest	30	60	90	120
Lactate (mM)	0.77 $\pm$ 0.08	1.29 $\pm$ 0.21*	1.03 $\pm$ 0.11*,†	0.93 $\pm$ 0.06*,†	0.96 $\pm$ 0.09*,†
Glycerol (mM)	0.04 $\pm$ 0.006	0.06 $\pm$ 0.006*	0.06 $\pm$ 0.009*	0.06 $\pm$ 0.008*	0.10 $\pm$ 0.012*

*Significant change when compared to rest. $p < 0.0346$ (experiment wise alpha < 0.05).

†Significant change when compared to Placebo $p < 0.0346$ (experiment wise alpha < 0.05).

Table 5. Changes in blood immune markers during the carbohydrate and placebo exercise trials. Expressed as mean $\pm$ SEM.

<u>time</u>	<u>Placebo</u>		<u>Carbohydrate</u>	
	Pre	Post	Pre	Post
White Blood Cells (1000 mm ³⁽⁻¹⁾)	4.95 $\pm$ 0.52	11.69 $\pm$ 0.89*	5.31 $\pm$ 0.35	11.21 $\pm$ 1.54*
Lymphocytes (1000 mm ³⁽⁻¹⁾)	1.63 $\pm$ 0.18	2.79 $\pm$ 0.47*	1.74 $\pm$ 0.24	2.56 $\pm$ 0.62*
Neutrophils (1000 mm ³⁽⁻¹⁾)	2.82 $\pm$ 0.48	7.98 $\pm$ 0.88*	2.96 $\pm$ 0.24	7.78 $\pm$ 1.27*
Monocytes (1000 mm ³⁽⁻¹⁾)	0.40 $\pm$ 0.04	0.77 $\pm$ 0.04*	0.46 $\pm$ 0.03	0.76 $\pm$ 0.11*
Eosophils (1000 mm ³⁽⁻¹⁾)	0.10 $\pm$ 0.02	0.11 $\pm$ 0.02	0.11 $\pm$ 0.03	0.07 $\pm$ 0.02

All values are corrected for changes in plasma volume.

*Significant change when compared to pre. $p < 0.037$ (experiment wise alpha < 0.05)

Figure Legends

- Figure 1.** Changes in plasma glucose during the 120 minute exercise trials. * $p < 0.0346$ (experiment wise alpha < 0.05) vs. placebo. † $p < 0.0346$ (experiment wise alpha < 0.05) vs. rest.
- Figure 2.** Changes in salivary flow rate during the 120 minute exercise trials.
- Figure 3.** Changes in s-IgA ($\mu\text{g/ml}$) during the 120 minute exercise trials. * $p < 0.035$ (experiment wise alpha < 0.05) vs. rest.
- Figure 4.** Changes in IgA secretion rate ($\mu\text{g/min}$) during the 120 minute exercise trials. * $p < 0.035$ (experiment wise alpha < 0.05) vs. rest.
- Figure 5.** Changes in protein secretion rate during the 120 minute exercise trials. * $p < 0.035$ (experiment wise alpha < 0.05) vs. rest. † $p < 0.0346$ (experiment wise alpha < 0.05) vs. placebo.
- Figure 6.** Changes in $\mu\text{g IgA/mg protein}$ during the 120 minute exercise trials. * $p < 0.035$ (experiment wise alpha < 0.05) vs. rest.