

THE EFFECT OF EXERCISING IN THE COLD ON MARKERS OF FLUID
BALANCE IN WOMEN

by

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ABSTRACT

The purpose of the study was to examine the effects of a cold temperature environment on markers of fluid balance in women during submaximal exercise. Nine women completed a 90-minute submaximal cycling protocol in both a cold (-5°C) and temperate (24°C) environment. The dependent variables were heart rate (HR), systolic and diastolic blood pressure (SBP, DBP), ratings of perceived exertion (RPE), percent change in plasma volume ($\%\Delta\text{PV}$), and percent change in body mass ($\%\Delta\text{BM}$). A two-way RMANOVA was used to detect differences over time and temperature condition. Over time, HR, SBP, and RPE increased during exercise irrespective of temperature environment, while DBP did not change significantly. Between condition, $\%\Delta\text{PV}$ and $\%\Delta\text{BM}$ were significantly lower in the cold environment. The combination of results indicates that water is shifting out of the plasma volume, but is then being restored after termination of cold exposure and exercise.

CHAPTER 1

INTRODUCTION

The role of hydration in exercise performance is an area of dynamic research within the field of exercise physiology. Proper hydration, for example, is an important factor in maximizing exercise performance, while dehydration is known to have detrimental effects on exercise performance (Oppliger et al. 2005).

Optimum hydration depends upon numerous of factors, including the environment and the menstrual cycle. For example, a warm environment accelerates water loss from the body through excessive sweating (Maughan et al. 2007; Shirreffs 2000). Thus, exercising in a warm environment requires special attention to maintain hydration status. While the effects of dehydration in a warm environment on exercise performance have been extensively studied (Fink et al. 1975; McFarlin and Mitchell 2003; Armstrong et al. 2010), research is needed on the effect of cold temperature on hydration and resulting exercise performance. Cold exposure imposes unique stresses on the human body, especially during exercise. In order to fully examine the effect of cold exposure during exercise on the body, underlying mechanisms need to be identified. Studies conducted on men have identified some components of the effect of hydration during exercise in the cold (Kennefick et al. 2004; Vogaelare et al. 1990). However, these studies have either failed to include women or account for the potential influence of the menstrual cycle. The menstrual cycle has a potential effect on hydration due to fluctuation of steroid hormones estrogen and progesterone. These hormones influence both water retention and

excretion. The effect of the menstrual cycle is difficult to control in hydration studies so is commonly ignored, or women are excluded from hydration studies altogether (Cheuvront et al. 2005; Vogaelare et al. 1990).

Understanding the effects of women's dehydration in the cold can potentially improve exercise performance. The effects of dehydration can be determined by observing differences in hydration status during exercise in a cold environment as compared to exercise in a warm environment. However, no studies have addressed this topic in women. Thus, the purpose of this study is to examine the effects of temperature on markers of hydration status during submaximal exercise in women.

Statement of the Problem

To investigate and describe changes in markers of hydration status for women exercising in the cold (-5°C) as compared to exercising in a temperate environment (24°C) during the follicular phase of the menstrual cycle.

Hypotheses

It was hypothesized that measures of percent change in plasma volume ($\%\Delta\text{PV}$), percent change in body mass ($\%\Delta\text{BM}$), heart rate (HR), and rating of perceived exertion (RPE) would be greater at all time points during exercise in the temperate environment as compared to the respective markers in the cold environment. It was also hypothesized that systolic (SBP) and diastolic (DBP) blood pressure would be lower at all time points

during exercise in the temperate environment as compared to the respective markers in the cold environment.

$$H_0: \mu_{C(i,j)} = \mu_{T(i,j)}$$

$$H_{A1}: \mu_{C(i)} < \mu_{T(i)},$$

$$H_{A2}: \mu_{C(j)} > \mu_{T(j)},$$

where μ_C and μ_T represent mean values in the cold environment and temperate environment, respectively. The subscript “i” represents the dependent variables expected to be a significantly higher magnitude of change at all time points in the temperate environment (% Δ PPV, % Δ BM, HR, RPE), whereas the subscript “j” represents the dependent variables expected to be lower at all time points in the temperate environment (SBP, DBP).

Significance of Study

In an effort to augment the existing literature, this study was designed to measure differences between fluid markers in cold (-5°C) and temperate (24°C) environments during submaximal exercise in women. The results of this study will further the understanding of how women react to submaximal steady-state exercise in the cold.

Delimitations

The study was delimited to women 18-45 years of age with regular menstrual cycles. Women who were amenorrhea, oligomenorrhic, or had premenopausal symptoms were excluded from the study.

Limitations

Subjects were recruited on a volunteer basis and received no compensation for their participation. Additionally, the study is limited to inference from indirect measures of hydration status.

Assumptions

It was assumed that women taking oral contraceptives have the same fluid regulation during their follicular phase as women who do not take oral contraceptives. It was also assumed that subjects answered all health history questions truthfully and adhered to dietary and exercise protocol restrictions.

Definitions

Diastolic Blood Pressure (DBP): Diastolic blood pressure is a measure of total peripheral resistance in the cardiovascular system during the relaxation phase of the heart, known as diastole, and is measured in millimeters of mercury (mmHg).

Hematocrit: Hematocrit is the red blood cell constituent of the blood and can be used to compute percent changes in plasma volume. Hematocrit is computed as a ratio of the volume of red cells to the volume of whole blood and is recorded as a percentage (%).

Hemoglobin: Hemoglobin is the oxygen transport protein portion of red blood cells and is also used to calculate percent changes in plasma volume in grams per deciliter (g/dl).

Maximal Oxygen Uptake (VO_{2MAX}): Maximal oxygen uptake (VO_{2MAX}) is the maximal rate at which muscle can utilize oxygen during exercise and is measured in Liters per minute (L/min), whereas relative VO_{2MAX} is measured in milliliters per kilogram per minute (ml/kg/min).

Systolic Blood Pressure (SBP): Systolic blood pressure is a measure of total peripheral resistance in the cardiovascular system during the contraction phase of the heart, known as systole, and is measured in millimeters of mercury (mmHg).

Urine Specific Gravity (USG): Urine specific gravity is a measure of the amount of particulates within a urine sample in units of grams per milliliter (g/ml).

Operational Definitions

Cold Environment: In the current study, a cold environment was defined as a mean ambient air temperature of -5°C .

Dehydration: Dehydration was defined as a measure of urine specific gravity of >1.01 g/ml. For the present study dehydration was defined as a reduction in body mass (kg) of one percent due to water loss during submaximal exercise (Oppliger et al 2005).

Euhydration: Euhydration was defined as a measure of urine specific gravity of <1.01 g/ml.

Follicular Phase: The follicular phase was defined as day one through ten of the menstrual cycle, where day one was defined as the first day of bleeding.

Hypohydration: Hypohydration was defined as a measured reduction in body mass (kg) of one percent due to water loss during submaximal exercise. Hypohydration is another term for dehydration.

Luteal Phase: The luteal phase was defined as day eleven through the end of menstrual cycle, where the end was the last day before bleeding.

Temperate Environment: In the current study, a temperate environment was defined as a mean ambient air temperature of 24°C.

Volitional Exhaustion: Volitional exhaustion was defined as the inability to sustain a pedaling cadence of 70 rpm for ten consecutive seconds at the end of a VO_{2MAX} test.

CHAPTER 2

REVIEW OF RELATED LITERATURE

Introduction

Hydration and its effect on exercise performance can be influenced by a number of factors including fluid regulation, environmental temperature and gender differences. Researchers use markers of hydration status to understand how fluid regulation is affected by exercise via changes in hormone and pressure levels. External temperature can influence thermoregulatory control which can also affect hydration status. Differences between genders are important to study because sex hormones can affect fluid regulation. However, hydration status in cold environments has not been researched as thoroughly in women as in men. Therefore, the purpose of this study was to investigate the effects of exercising in the cold on markers of fluid balance in healthy women during the follicular phase.

Fluid Regulation

Fluid Compartments and Movement

Fluid dynamics describe how fluid moves between the three fluid compartments of the body: the intracellular, extracellular, and vascular spaces. The intracellular space accounts for half of the fluid in the body, between 20-25 liters, and is confined within the cell (Davy and Seals 1994). Fluid in the intracellular space assists with particle mobilization and keeps an inherent pressure in the cell. The extracellular space is the

second largest fluid compartment, around 18 liters, and consists of everything outside of the cell. Fluid in the extracellular space bathes surrounding tissues and acts as a medium between cells (Davy and Seals 1994). The vascular space, or blood volume, is a specialized extracellular space, specific to the cardiovascular system. The fluid portion of the blood volume, around 3 liters, is known as the plasma volume. The plasma volume, fluctuates according to the concentration of blood solids that are both inside and outside of the vascular space (Kurbel et al. 2001)

Fluid moves between compartments by changes in pressure. This pressure can be hydrostatic, osmotic, or oncotic pressure. Hydrostatic fluid pressure is the effect of gravity or any accelerating force on the fluid compartments of the body. Osmotic pressure moves water in response to solute concentrations, specifically glucose and ion gradients. Water will passively flow from a low particle concentration to a high particle concentration. Oncotic pressure is a specific type of osmotic pressure, in which water moves in response to a protein concentration gradient (Kurbel et al. 2010).

Hormones that Influence Fluid Balance

Movement of fluid and changes in pressure between compartments can be regulated by hormones acting within the body. The principle fluid regulating hormones are anti-diuretic hormone (ADH), aldosterone (ALD), and atrial natriuretic hormone or factor (ANF). Fluctuations of these hormones will cause either water retention or absorption.

The primary function of ADH is to retain water in the body. An increase in osmolality outside of the fluid homeostatic range signals a release of ADH from the

posterior pituitary gland. Anti-diuretic hormone then attaches to receptors in the distal convoluted tubules of the kidneys, signaling for retention of water (Exercise Physiology 2005).

Aldosterone is a sodium regulating hormone which effectively regulates water retention. Sensors at the macula densa within the kidneys monitor sodium levels. When the body becomes dehydrated, angiotensin stimulates the adrenal glands to release aldosterone. Aldosterone signals the distal convoluted tubules of the kidney to reabsorb sodium which, due to osmotic pressure, also causes water to be reabsorbed (Exercise Physiology 2005).

Atrial natriuretic hormone regulates fluid in response to increases in central venous pressure. As central venous pressure increases, diastolic and systolic pressures increase, resulting in greater stress on the heart. In response to the greater stress, ANF is released to stimulate an increase in urine output. As urine is excreted from the body, blood volume is reduced, resulting in a decrease in central venous pressure which lessens stress on the heart. During cold exposure the body redirects blood from the periphery to core through vasoconstriction of blood vessels. As blood is redirected to the core, central venous pressure increases, which in turn stimulates the release of ANF (Legault et al. 1992). In summary, the hormones, ADH, ALD, and ANF are affected by exercise through disruption of fluid homeostasis, while markers of hydration status can be used to monitor fluid homeostasis.

Measures of Fluid Regulation and Cardiovascular Strain

Fluctuations in the control of fluid homeostasis can be assessed by markers of hydration status. As the body dehydrates, changes in body mass, plasma volume, blood pressure, and ratings of perceived exertion can be measured. Each measure is related to a physiological process occurring in response to body water loss.

Measuring body mass over the course of a dehydration protocol is a simple and accurate way to measure water loss. Water loss between 1% and 3% of body weight is classified as minimal dehydration, whereas greater than 5% is extreme dehydration associated with heat stroke and death (Oppliger et al. 2005).

Percent change in plasma volume is a measure of the body's ability to pull fluid from the intracellular space to replenish water loss via exhalation and sweating (Jimenez et al. 1999). Percent change in plasma volume can indirectly be measured over the course of a dehydration protocol via measures of hematocrit and hemoglobin concentration (Greenleaf and Convertino 1979). The use of hematocrit and hemoglobin has been shown to provide accurate measures of percent change in plasma volume (Jimenez et al. 1999).

Plasma volume is calculated using the equations from Dill and Costill (1974):

$$\Delta BV (\%) = [(BV_A - BV_B)/BV_B] \times 100$$

$$\Delta CV (\%) = [(CV_A - CV_B)/CV_B] \times 100$$

$$\Delta PV (\%) = [(PV_A - PV_B)/PV_B] \times 100$$

where, subscript A is the reference measure, subscript B is the end measure of the interval being observed, ΔBV is change in blood volume, ΔCV is change in cellular

volume, and ΔPV is change in plasma volume. These equations were used to by Harrison et al. (1982) to derive the following equation:

$$\% \Delta PV = ((Hb_C / Hb_T) \times ((100 - HCT_T) / (100 - HCT_C)) - 1) \times 100,$$

where, the subscript “C” represents the control measure and the subscript “T” represents the second measure. A control measure is used to establish a baseline value to which the second measure can compare. This equation is then used to calculate percent changes in plasma volume using hematocrit and hemoglobin measurements. The Harrison equation is best suited for studies using the direct measures of hematocrit and hemoglobin to indirectly measure percent change in plasma volume. The Harrison equation allows for greater ease of measuring plasma volume and less invasiveness toward the subject.

In addition to hematocrit and hemoglobin, blood pressure is another marker of hydration status. Blood pressure changes in response to internal and external stimuli. As internal temperature increases blood vessels vasodilate. Vasodilation increases the radius of the vessels and results in a reduction in mean arterial pressure (Maughan et al. 2007). An example of an external stimulus is the effect of a cold environment on blood pressure. As the external temperature decreases, blood vessels are vasoconstricted in the periphery, which redirects blood flow to the central system, and increases systolic and diastolic blood pressures (Legault et al. 1992). Therefore, monitoring blood pressure during an exercise protocol in a cold environment will provide insight into which stimuli, external or internal, is dominant.

A qualitative measure that describes how the participant is feeling in response to dehydration is known as the rating of perceived exertion, or RPE. For this measure, a

modified Borg scale is used to refer to the whole body feeling of the participant via a numerical scale (Aliverti et al. 2011; Borg and Kaijser 2006; Borg et al. 2010). Borg and Kaijser (2006) found that when performing graded exercise tests on a cycle ergometer, there was a strong correlation ($r= 0.98$) between RPE on the CR10 modified Borg scale and heart rate values. The authors concluded that the modified Borg scale was well suited to studies using cycle ergometry.

Fluid Regulation and the Menstrual Cycle

Menstrual Cycle Components

Fluid balance research on women is difficult due to the influence of the fluctuating sex steroids levels during the menstrual cycle. The menstrual cycle lasts an average of 28 days and is divided into the follicular and luteal phases. The follicular phase begins on day one of the menstrual cycle and ends at ovulation, whereas the luteal phase begins at ovulation and ends at menstruation. Sex hormones, estrogen and progesterone, fluctuate throughout the menstrual cycle. Estrogen concentration, for example, is low during the follicular phase, followed by a spike at ovulation, and then continues to increase during the luteal phase. In contrast, progesterone concentration is low until the luteal phase then increases until menstruation (Exercise Physiology 2005).

Effects of Sex Hormones on Fluid Regulation

Estrogen and progesterone have the greatest effect on fluid regulation during the luteal phase of the menstrual cycle. High concentrations of estrogen and progesterone can stimulate water retention during the luteal phase of the menstrual cycle. Given that

changes in water retention due to these hormones can confound the study of fluid balance in women, fluid balance research on women is typically performed during the follicular phase of the cycle when progesterone and estrogen levels are lowest (Calzone et al. 2001; Stachenfeld et al. 2002; Stachenfeld et al. 1999). Thus, the follicular phase of the menstrual cycle appears to be the best stage to examine the relationship between cold exposure and exercise.

Effects of Oral Contraceptives on Fluid Regulation

The use of oral contraceptives can confound measures of fluid balance during the luteal phase of the menstrual cycle (Stachenfeld et al. 1999). For example, research has been conducted on the effect of oral contraceptives and sex steroids on thermoregulation during submaximal exercise at 50% of VO_{2MAX} on a cycle ergometer. Gruzca et al. (1993) found that women not taking oral contraceptives had an upward shift in core temperature by $0.23^{\circ}C$ during the luteal phase which corresponded to an increase in sweat output. Women in the same study taking oral contraceptives still showed a $0.25^{\circ}C$ increase in core temperature during the luteal phase, but no increase in sweat production. Temperature and sweat rate remained similar between groups in the follicular phase. Thus, if women are taking oral contraceptives it is best to evaluate measures of hydration status during the follicular phase of the menstrual cycle.

Fluid Regulation During Exercise in Hot and Cold Environments

The Effects of a Hot Environment and Exercise on Fluid Regulation

A hot environment imposes thermal stresses on the body that require the activation of heat dissipation mechanisms. The body dissipates heat via convection, conduction, radiation, and evaporation. Heat loss by radiation is best achieved by redirecting blood flow using blood vessel vasodilation and vasoconstriction.

Vasodilation of the blood vessel moves the blood vessel closer to the skin surface where heat can dissipate via radiation. Since blood is drawn away from working muscle when blood vessels vasodilate, the cardiovascular system has to increase cardiac output to maintain both heat dissipation and muscle blood flow during physical activity.

During exercise chemical reactions are taking place in the contracting muscles. Of the energy produced in these reactions, seventy-five percent comes in the form of thermal energy or heat. In order to dissipate the heat, the hypothalamus redirects blood flow to the skin surface to allow heat to transfer to the external environment via radiation (Exercise Physiology 2005).

Research on the effects of external temperature on exercise performance has been extensive. Fink et al. (1975) examined the effects of exercising in 41°C and 9°C environments on physiological functions. The authors found that oxygen uptake, rectal temperature, and heart rate were all significantly higher in the hot environment as compared to the cold environment. The authors also found a two-fold increase in lactate production in the hot environment and a concurrent increase in glycogen utilization. The

authors suggested that greater cardiovascular strain was exhibited in a hot environment due to reduced muscle blood flow. The authors' conclusions concur with results from exercise related studies in temperate and hot environments (Galloway and Maughan 1998; McFarlin and Mitchell 2003). Galloway and Maughan (1998) found that there was an increase in cardiovascular strain in a temperate environment during submaximal exercise to exhaustion, but no change in cardiovascular strain in the cold. Additionally, McFarlin and Mitchell (2003) concluded that exercising in the heat elicited greater physiological stress than exercising in the cold environment.

The Effects of Cold Environment and Exercise on Fluid Regulation

The mechanisms for thermoregulation in the cold differ from those observed in temperate or hot environments. At rest, in a cold environment, the body draws blood away from the periphery and redirects it to the core and neck regions in an effort to retain heat and warm inspired air (McFadden et al. 1999). The redirection of blood flow by peripheral and central vasoconstriction increases systolic and diastolic pressure (Therminarias 1992). In contrast, during continuous and interval exercise in the cold, Muller et al. (2010) found that peripheral blood flow was maintained. Muller and colleagues postulated that an exercise induced increase in internal temperature countered blood flow to the core by increasing blood flow to the periphery.

The relationship between heat retention in the cold and heat production during exercise is further addressed in the research literature. Research conducted by Vogelaere et al. (1990) examined men's hematological variations at different exercise intensities in

the cold (0°C). Vogelaere et al. found that cold stress induced an increase in red blood cell derivatives (hemoglobin and hematocrit) in all test conditions. Concurrently, the researchers found a -7.31% percentage change in plasma volume ($\Delta\%PV$) at room temperature and a -9.31% at 0°C after 120 minutes of submaximal exercise. The authors suggested that these results were indicative of fluid shifts between the intracellular and extracellular compartments. Plasma volume was reduced and water exited the extracellular space and possibly shifted into the intracellular space. It was concluded that hematological variables were affected by cold exposure, even under exercise conditions.

Kennefick et al. (2004) examined the effect of dehydration on thermoregulatory responses in cold and hot environments. Thermoregulatory and cardiovascular strain did not increase during exercise in the cold environment even when the subjects were dehydrated. The authors concluded that internal heat production had a stronger influence on fluid shifts in comparison to the cold environment. The cold environment, however, allowed heat to dissipate fast enough that cardiac drift did not occur. This finding is opposite to that observed in the heat, where thermoregulatory and cardiovascular strain would increase under hypohydrated conditions. This is further supported by Maw et al. (1998) findings of that after a 50-minute cycling protocol, blood plasma volume was maintained in cold and temperate environments, thus reducing cardiovascular strain. Additional research was conducted on the performance benefits of carbohydrate and fluid ingestion during cycling. Galloway and Maughan (1998) studied the effect of carbohydrate concentration in fluid ingestion on exercise performance during submaximal exercise in the cold. Concentration of carbohydrate had significant effects

on performance levels and water retention in the warm environment, but no effect in the cold. It was concluded that cold stress was low enough that carbohydrate supplementation was not needed.

In summary, the relationship between cold exposure and exercise is characterized by lower cardiac strain, greater percent changes in plasma volume, and shifts in concentration of hematological variables, in men. Little research, however, examines these relationships in women.

Summary

Fluid moves throughout the body through a network of compartments via pressure and hormone regulation. As humans exercise, shifts in fluids occur relative to muscle contraction, heat production, as well as fluid and food intake. Environmental temperature can be a strong influence on fluid dynamics as blood flow is moved to either the periphery or to the core during hot and cold exposure, respectively. Exercise can further complicate the fluid response in the body. Indeed, exercise can give rise to different physiological responses when coupled with a hot or cold environment. These responses have been investigated in the male population, but have yet to be addressed as thoroughly in women. Therefore, the purpose of this study was to investigate the effects of exercising in the cold on markers of fluid balance in healthy women during the follicular phase.

CHAPTER 3

THESIS MANUSCRIPT

Introduction

The role of hydration on exercise performance, especially the ability to maintain optimum hydration, is an area of research within the field of exercise physiology. The ability to maintain the correct level of hydration during exercise can lead to performance benefits. Optimum hydration depends upon a variety of factors which include ambient temperature, relative humidity, training status, drinking habits during exercise, exercise intensity, amount of clothing worn during exercise, and the menstrual cycle for women. It is important then to examine the effects of submaximal exercise on hydration status in a cold environment during the follicular phase of the menstrual cycle.

The effects of dehydration in warm environments on exercise performance have been thoroughly explored (Fink et al. 1975; McFarlin and Mitchell 2003; Armstrong et al. 2010). Still to be elucidated is the effect of a cold environment on hydration and resulting exercise performance. Cold exposure imposes unique stresses on the human body, especially during exercise. Studies have identified some components of how cold environments affect hydration status during submaximal exercise (Kennefick et al. 2004; Vogaelare et al. 1990). However, these studies did not include women or failed to account for the menstrual cycle. Thus, there is a lack of well-designed research examining the effect of exercising in the cold on hydration in women.

Understanding the effects of dehydration in the cold can potentially increase exercise performance. Specifically, how markers of dehydration change in a cold exercise environment in comparison to a temperate exercise environment should be tested. The purpose of this study, therefore, was to measure fluid regulation markers during a dehydration protocol and compare responses from both cold (-5°C) and temperate (24°C) environments. The measures of hydration status used for this study were percent change in plasma volume ($\%\Delta\text{PV}$), percent change in body mass ($\%\Delta\text{BM}$), heart rate (HR), rating of perceived exertion (RPE), and systolic and diastolic pressure (SBP, DBP). The hypothesis was that $\%\Delta\text{PV}$, $\%\Delta\text{BM}$, HR, and RPE would be higher at all time points during exercise in the temperate environment. Additionally, it was hypothesized that SBP and DBP would be lower at all time points in the temperate environment. These hypotheses are congruent with those found in studies on men under similar exercising conditions (Kennefick et al. 2004; Vogalaere et al. 1990).

Methods

Subjects

Women between the ages of 18 and 45 years, who were self-reported habitually physically active, were recruited from Montana State University and the greater Bozeman area. All subjects, as determined by one-on-one interview with primary investigator, had regularly occurring menstrual cycles for three months prior to testing. Women having self-reported amenorrhea, oligomenorrhea, or premenopausal symptoms were excluded from the study. All of the subjects completed a health history questionnaire prior to

testing to identify contraindications to maximal or submaximal cycle testing (American College of Sports Medicine 2010). Prior to participation, subjects read and signed an informed consent document approved by the Montana State Institutional Review Board (Appendix A).

Procedures

Subjects performed three testing sessions for the study. Upon the first day of menses, subjects were instructed to contact the principal investigator for scheduling. To mitigate the effects of estrogen and progesterone on fluid regulation, subjects were tested during the first ten days of the menstrual cycle when these hormone concentrations should be lowest. The first session occurred between days 5 and 7 of the menstrual cycle. The second session commenced a minimum of two days after Session 1, but no later than day 10 of the menstrual cycle, while Session 3 occurred one month later on the same day of the menstrual cycle as Session 2.

Session 1 Protocol

At Session 1, the subjects' body height and mass were assessed prior to a maximal oxygen uptake (VO_{2MAX}) test on a cycle ergometer. Metabolic demands were assessed using measurements of oxygen consumption (VO_2) and carbon dioxide production via indirect open circuit spirometry with a metabolic measurement system, reporting 20-second sample averages.

VO_{2MAX} Test Protocol

Subjects began with a five-minute warm-up on a cycle ergometer at a power output of 37 Watts. The VO_{2MAX} test began at a power output of 74 watts and 70 RPM for three minutes, where each stage thereafter increased by 23 watts at the same RPM until volitional exhaustion. Measurements of fingertip blood lactate and heart rate were collected and recorded in the last minute of each 3-minute stage. An absolute measurement of blood lactate above 5 mmol, or a measurement of blood lactate 2 mmol higher than the previous stage, was used to indicate lactate threshold. Once, lactate threshold was observed, stage duration was reduced from three to one minute, heart rate continued to be monitored, but blood lactate measurements ceased. The test concluded when the subject no longer maintained the 70 rpm cadence or until volitional exhaustion.

Maximal oxygen uptake was taken as the single highest 20-second VO₂ value so long as the two of three criteria were satisfied: 1) Respiratory exchange ratio (RER) of ≥ 1.1 ; 2) Maximum observed heart rate was within of ± 10 BPM of age-predicted maximal heart rate; 3) Highest successive VO₂ measures were within ± 2.5 ml/kg/min at the end of the test.

Upon successful completion of the VO_{2MAX} test, lactate values were used to compute power output at lactate threshold (P_{LT}, W). Values for P_{LT} were calculated by plotting lactate values in response to time and fitting two lines to the data points. Lines were fit according to visual observation of the data, where the first line represented the slope of the first two data points prior to increase in lactate values and the second line represented the slope of the last two data points after an increase in lactate values were

evident. The intersection of the two lines was determined to be the lactate threshold. This value was then used as a basis for setting cycling power output in subsequent testing sessions so that each cyclist began Sessions 2 and 3 at a power output approximating 90% of P_{LT} .

Protocol for Sessions 2 and 3

During session 2, subjects performed a 90-minute cycling protocol in either a cold environment (-5°C) in the Montana State University Subzero Science and Engineering Research Facility, or a temperate environment (24°C) in the Montana State University Movement Science Laboratory. The subjects were randomly assigned a counterbalanced order to their initial environmental condition for session 2, with the opposite condition assigned for session 3. The cycling protocol description was segmented into pre-session, pre-cycling, cycling, and post cycling sections.

Subjects were asked to abstain from alcohol, caffeine, and exercise the day before and on the day of the session. Additionally, subjects recorded a 24 hour diet log prior to session 2 testing. The diet log was used as a reference for food consumption prior to session 3 testing. Subjects drank a minimum of 300 milliliters of water one hour prior to the session to establish euhydration, which was verified using urine specific gravity on a urine sample collected in the lab. For testing in the cold, subjects dressed in athletic sweats, long-sleeved base layer, gloves, socks, hat, and appropriate footwear. All cold testing clothing was provided by the principle investigator, while subjects provided their own footwear. For temperate testing, subjects provided their own shorts, t-shirt, socks, and appropriate footwear.

Upon arrival, the subjects provided a urine sample which was tested for urine specific gravity, to ensure urine samples were less than or equal to 1.01 g/ml (Oppliger et al. 2005). Subjects were then measured for nude body mass and outfitted with a heart rate monitor that was worn under the bra line. Subjects sat for five minutes before measuring resting blood pressure and heart rate, where blood pressure was measured on the left wrist using a wrist blood pressure cuff. Fingertick blood samples were also taken on the right hand to assess levels of hemoglobin and hematocrit. Once these measurements were complete, subjects commenced with the cycling protocol.

Subjects performed a 10-minute warm-up and then began the 90-minute continuous dehydration cycling protocol, with the exception of one-minute breaks after cycling for 30 and 60 minutes. The breaks were used to alleviate subject discomfort due to sustained pressure by the seat of the cycle ergometer. Immediately prior to each break, measures of blood pressure (BP), fingertick blood collection, rating of perceived exertion (RPE), and heart rate (HR) were taken. Subjects remained in a seated, upright posture, five minutes prior to blood pressure measurement, to ensure fluid compartment stabilization. The intensity of the protocol was set at 90% of subject P_{LT} as determined from Session 1. Additionally, subjects abstained from drinking any liquid during the entire 90-minute session.

After dismounting the cycle ergometer, the subject's nude body mass was recorded and a urine sample collected. Subjects then sat for fifteen minutes in the temperate environment to allow for fluid compartment stabilization, after which, final

measurements of blood pressure, heart rate, and a fingerstick blood sample were collected.

Instrumentation

Cycle testing was administered with a cycle ergometer (Monarck 828E, Sweden). Metabolic data were collected using standard open circuit spirometry data acquisition hardware and software (TrueMax 2400, ParvoMedics, Sandy, UT, USA). The metabolic system included a mouthpiece with two one-way valves, a hose for the collection of expiratory gases, and a nose clip. Flow rates and gas concentrations were calibrated using a 3-L syringe (Series 5530, Hans Rudolph, Kansas City, MO, USA) and known concentration calibrations gases prior to each test session, respectively. Body mass was measured on an electronic scale to the nearest 0.1 kg (BWB-800S, Tanita Corporation, Tokyo, Japan), while heart rate was measured during maximal and submaximal testing using a Polar heart rate chest strap and watch (RS400, Polar Heart Rate Monitor, Kempele, Finland). Two blood samples were taken each measurement period and analyzed using a HemoPoint H₂ analyzer (DMS, Stanbio Laboratory, Boerne, TX, USA), the results of which were averaged (Conder et al. 2011).

Data Processing

Values of % Δ PV were calculated using the equation developed by Harrison et al. (1982):

$$\% \Delta PV = ((Hb_C / Hb_T) \times ((100 - HCT_T) / (100 - HCT_C)) - 1) \times 100$$

where Hb represents hemoglobin concentration (g/dL), HCT represents hematocrit (%), the subscript “C” represents the control measure and the subscript “T” represents the second measure. For this study, the control measure was defined as the resting value prior to exercise and the second measure was that obtained at 30-minutes, 60-minutes, 90-minutes, or post exercise (Pre30, Pre60, Pre90, PrePost respectively). Change scores were also calculated for heart rate, systolic blood pressure, and diastolic blood pressure, where resting values were defined as the same intervals Pre30, Pre60, Pre90, and PrePost.

Statistical Analyses

Multivariate two-factor (environment by time) repeated measures analysis of variance (RMANOVA) at the 0.05 alpha level were used to detect differences for body mass, Δ APV, systolic (SBP) and diastolic blood pressure (DBP), RPE, and heart rate. Dunnett’s 2-sided Multiple Comparison Test was used for post hoc analyses at the 0.05 alpha level to compare the two environmental conditions at discrete time points 0, 30, 60, 90 minutes, and post test, with the 0-minute time point being the reference value (TPre, T30, T60, T90, and TPost, respectively).

Results

A total of 10 subjects participated in the study, but only nine subjects completed both temperate and cold trial conditions. Demographics for the nine subjects who completed the study are summarized in Table 3.1.

RMANOVA Analyses:

A statistically significant main effect for time was detected for HR, SBP, DBP, RPE, and $\% \Delta PV$. Additionally, a statistically significant main effect for condition was found for $\% \Delta PV$ and $\% \Delta BM$.

Mean exercising HR values (Table 3.2) at T30, T60, and T90 were significantly higher ($P < 0.05$) than pre-exercising HR (TPre), while post-exercise (TPost) HR values were statistically similar. There were no differences in mean HR values between conditions. Delta heart rate (ΔHR) values (Table 3.3) were identical to that for HR. Both HR and ΔHR values had a non-significant tendency to have higher Tpost values during the temperate condition. Mean SBP values (Table 3.2) at T60 and T90 were significantly higher ($P < 0.05$) than TPre, while values at T30 and TPost were similar. There were no differences in mean SBP values between conditions. Delta systolic blood pressure (ΔSBP) values (Table 3.3) were identical to that of SBP. Mean DBP and ΔDBP values did not differ significantly across time or condition. While a main effect for time was detected by the ANOVA, post-hoc analyses did not detect any differences, which was due to either a lack of difference or low sample size. Mean RPE values (Table 3.2) at T30, T60, and T90 were significantly higher ($P < 0.05$) than TPre, while TPost values were similar. There were no differences in mean RPE values between conditions. Mean $\Delta \% PV$ values (Table 3.4) were significantly higher ($P < 0.05$) at Pre60 in the temperate condition. Mean $\Delta \% PV$ values were trending toward significantly higher ($P < 0.10$) at Pre90 in the temperate condition. Mean $\Delta \% BM$ values (Table 3.5) were significantly

less ($P < 0.01$) in the cold condition than in the temperate. There was no significant difference ($P = 0.34$) in urine output (Table 3.5) between conditions.

Table 3.1. Measurements taken of individual subject characteristics.

Subject #	Body Height (cm)	Body Mass (kg)	VO _{2max} (L/min)	VO _{2max} (ml/kg/min)	BMI (kg/m ²)	Power Output (W)
1	164.7	61.7	2.34	37.9	22.7	103.3
2	165.7	73.6	2.58	35.1	26.8	103.3
3	171.5	67.7	1.97	29.1	23.0	86.1
4	166.5	65.6	2.73	41.6	23.7	114.7
5	164.0	53.5	1.89	35.4	19.9	68.9
6	168.7	72.0	2.48	34.5	25.3	103.3
7	169.7	67.7	2.29	33.9	23.5	86.1
8	162.4	52.5	2.54	48.4	19.9	103.3
10	171.8	72.2	2.67	36.9	24.5	103.3
Mean	167.2	65.2	2.39	37	23.3	96.9
SD	3.4	7.9	0.3	5.4	2.3	13.9

Note: Subject #9 did not complete both trial conditions, therefore all data for subject was excluded from statistical analyses, SD=standard deviation. All subjects had urine specific gravity (USG) levels of ≤ 1.01 g/dL prior to starting submaximal exercise. Table 3.2. Summary of values for heart rate, systolic and diastolic blood pressure, as well as ratings of perceived exertion (Mean \pm SE).

Condition	Measure	TPre	T30	T60	T90	TPost
<u>Temperate</u>	HR (bpm)	76 \pm 4	159 \pm 5*	161 \pm 5*	162 \pm 4*	102 \pm 7
	SBP (mmHg)	111 \pm 3	123 \pm 4	118 \pm 5*	120 \pm 5*	101 \pm 3
	DBP (mmHg)	72 \pm 3	74 \pm 4	69 \pm 3	73 \pm 4	62 \pm 3
	RPE	0 \pm 0	4 \pm 0.4*	4 \pm 0.3*	4 \pm 0.5*	0 \pm 0
<u>Cold</u>	HR (bpm)	82 \pm 4	157 \pm 4*	160 \pm 2*	160 \pm 3*	87 \pm 4
	SBP (mmHg)	110 \pm 3	116 \pm 7	122 \pm 6*	128 \pm 5*	106 \pm 3
	DBP (mmHg)	70 \pm 3	74 \pm 4	77 \pm 4	78 \pm 4	71 \pm 3
	RPE	0 \pm 0	3 \pm 0.4*	4 \pm 0.4*	4 \pm 0.4*	0 \pm 0

Note: Dependent measures varied significantly across times if denoted with an *, HR=heart rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; RPE=ratings of perceived exertion; SE=standard error; TPre=time point at rest; T30=time point at 30-minutes of exercise; T60=time point at 60-minutes of exercise; T90=time point at 90 of exercise, TPost=time point at 15-minutes after exercise.

Table 3.3. Summary of values for delta heart rate, systolic and diastolic blood pressure, as well as ratings of perceived exertion (Mean±SE).

Condition	Measure	ΔPre30	ΔPre60	ΔPre90	ΔPrePost
<u>Temperate</u>	ΔHR (bpm)	83±4*	85±5*	86±4*	26±5
	ΔSBP (mmHg)	12±3	7±4*	9±4*	-9±4
	ΔDBP (mmHg)	2±2	-3±1	1±3	-10±3
<u>Cold</u>	ΔHR (bpm)	75±3*	77±43*	78±3*	6±3
	ΔSBP (mmHg)	6±6	11±7*	17±5*	-4±3
	ΔDBP (mmHg)	3±3	6±4	8±3	1±2

Note: Dependent measures varied significantly across times if denoted with an *, ΔHR=delta heart rate; ΔSBP=delta systolic blood pressure; ΔDBP=delta diastolic blood pressure; SE=standard error; ΔPre30=time interval between rest and 30-minutes of exercise; ΔPre60=time interval between rest and 60-minutes of exercise; ΔPre90=time interval between rest and 90-minutes of exercise; ΔPrePost=time interval between rest and 15-minutes after exercise.

Table 3.4. Summary of values for percent change in plasma volume (Mean±SE).

Condition	Measure	ΔPre30	ΔPre60	ΔPre90	ΔPrePost
<u>Temperate</u>	%ΔPV	-9.9±3.1	-6.5±3.0**	-8.4±2.8	-0.9±2.9
<u>Cold</u>	%ΔPV	17.2±3.1	-20.0±1.1**	-19.3±1.3	-4.1±2.1

Note: Dependent measures varied significantly across temperature conditions if denoted with an **, %ΔPV=percent change in plasma volume; SE=standard error; ΔPre30=time interval between rest and 30-minutes of exercise; ΔPre60=time interval between rest and 60-minutes of exercise; ΔPre90=time interval between rest and 90-minutes of exercise; ΔPrePost=time interval between rest and 15-minutes after exercise.

Table 3.5. Summary of values for body mass and urine output (Mean±SE).

Condition	Measure	TPre	TPost
<u>Temperate</u>	Body Mass (kg)	65.3±2.5	64.5±2.5**
	Urine Output (mL)		287±51.0
<u>Cold</u>	Body Mass (kg)	65.5±2.5	64.9±2.5**
	Urine Output (mL)		325±55

Note: Dependent measures varied significantly across temperature condition if denoted with an *, SE=standard error; TPre=time point at rest; TPost=time point at 15-minutes after exercise.

Discussion

While there have been numerous studies examining the role of environmental temperature on markers of hydration status in men and women, this is the first study, to the author's knowledge, that examines the effect of cold exposure on markers of hydration status in women. The purpose of this study was to measure changes in hydration status in women during submaximal exercise in the cold. The results of this study provide direct measures of women's responses to exercise in a cold environment.

The hypotheses were that % Δ PV, % Δ BM, HR, and RPE would be higher at all time points during exercise in the temperate environment. Additionally, it was hypothesized that SBP and DBP would be lower at all time points in the temperate environment. Percent change in plasma volume and percent change in body mass varied by temperature condition during submaximal exercise. Plasma volume decreased (6.5% \pm 3%) during 60 minutes of submaximal exercise in the temperate environment and decreased (20.0% \pm 1.0%) in the cold environment at the same time point. Percent change in body mass was significantly lower for the cold condition when compared to the

temperate condition, while urine output was not significantly different between conditions. These observations suggest that body mass reduction was greater in the temperate environment primarily due to an increased amount of sweat loss during the submaximal exercise. No significant difference was observed between conditions for the other variables measured in this study.

The significant decrease in percent change in plasma volume in women has also been observed in men. Vogelaere et al. (1990) found that after exercising in the cold, men had a greater reduction in percent change in plasma volume than in the temperate environment. In Vogelaere's study, male subjects performed a submaximal cycle ergometer protocol in cold (0°C) and temperate (20°C) environments. After cycling for 120 minutes at 40% of maximal power output, there was a decrease in plasma volume of 7.13% in the temperate environment and 9.31% in the cold environment. Vogelaere et al. concluded that the greater reduction in plasma volume in the cold was due to cold-induced resting plasma variations. The results of the current study support this conclusion. The combination of a high negative change in plasma volume and a smaller decrease in body mass in the cold condition over that observed in the temperate condition suggests that in cold conditions water shifts away from the plasma volume, which is transient in a cold environment. Upon returning to a temperate environment, fluids shift back to their original compartments.

These results have not been observed universally, even in testing performed on male subjects. In another study, Maw et al. had subjects cycle for 50 minutes at 50% of maximal power output. Maw et al. (1998) had each subject ingested radioactive nuclei

that tracked fluids shifted between the plasma volume, red cell volume, blood volume, intracellular water, extracellular-intracellular water, extracellular fluid volume, interstitial fluid volume, and total body water. Maw et al. (1998) found that after an initial reduction of plasma volume at 10 minutes of submaximal exercise, plasma volume recovered after 30 minutes of submaximal exercise in cool and temperate conditions (14.4°C and 22°C, respectively). The authors explained that the initial reduction in plasma volume was due to both an increased intravascular hydrostatic pressure and an elevated intramuscular osmotic force. Additionally, the recovery of plasma volume was due to increased interstitial hydrostatic pressure and increased plasma tonicity which combined to create an osmotic gradient favoring plasma volume restoration.

One possible reason for the conflicting results between the current study and Maw et al. is the temperature for the cold condition. In Maw et al. the temperature was 14.4°C for the cool environment, whereas -5°C was used for the current study. The study performed by Vogelaere et al., which resulted in a cold-induced water shift at all exercise levels, also tested in a much colder temperature of 0°C. It is possible that Maw et al. did not have a cold enough temperature to elicit the cold-induced water shift. Though it is difficult to determine precisely what temperature is necessary for this phenomenon to occur, it appears that it does require a cold temperature of less than 14°C.

Another possible reason for the difference in results could be due to catecholamine response during exercise in the cold. It is possible due to exercise and cold exposure that catecholamine levels increased, resulting in vasoconstriction in the periphery. This peripheral vasoconstriction could then alter the amount of blood flow to

the hands where the fingerstick blood sample was taken. If this was the case, plasma volume could have been artificially reduced in periphery.

Additional hypotheses were that HR and RPE would also be higher in the temperate environment when compared to the cold environment and that SBP and DBP would be lower in the temperate environment. The results were statistically similar across conditions for all of these variables. However, these same variables did change over time.

Markers of hydration status were observed to change over time during the 90 minutes of submaximal exercise. Heart rate increased during exercise and remained slightly elevated post exercise. Additionally, systolic blood pressure was elevated significantly above resting levels at 60 and 90 minutes into exercise. Finally, rating of perceived exertion (RPE) was also observed to be higher during exercise. These results are consistent with published research on the effects of continuous submaximal exercise on quantitative and qualitative cardiovascular measures and markers of hydration status (Maughan et al. 2007; Maw et al. 1998; Vogalaere et al. 1990).

Conclusions

In the present study, changes in markers of hydration status during submaximal exercise in women were similar to those reported in studies conducted on men. The current study did not find any significant differences between temperature conditions for hydration markers of heart rate (HR), systolic and diastolic pressures (SBP, DBP), and rating of perceived exertion (RPE). However, trends in data for HR and pressure

responses could indicate fluid shifts occurring solely in the cold environment. Heart rate and SBP values had higher post exercising values in the cold as compared to the temperate environment. A possible explanation for the trends could be due to cold induced vasoconstriction of the peripheral arterial system resulting in blood shunting away from the periphery toward the body's core, which would increase central pressure (both DBP and SBP) and HR. Additionally, the percent change in plasma volume in women was found to decrease with a greater magnitude in a cold environment than in a temperate environment. This result is similar in the direction that $\% \Delta PV$ changed in comparison to other published research on men (Vogalaere et al. 1990). Additionally, body mass was found to decrease less in a cold environment than in a temperate environment. These results indicate that temperature had a negative directionality effect on plasma volume fluctuation and fluid distribution during submaximal exercise in the cold. However, the results do not indicate if there is a difference in the magnitude of change in fluid distribution between men and women in a cold environment. A way to answer the question of magnitude would be to design a study that utilizes men and women, with men acting as the control group.

CHAPTER 4

CONCLUSIONS

A dehydration protocol conducted in both temperate and cold environments was used to determine shifts in markers of hydration status during submaximal exercising in women. Hydration status was monitored using percent change in plasma volume ($\% \Delta PV$), percent change in body mass ($\% \Delta BM$), heart rate (HR), systolic and diastolic pressure (SBP, DBP), and rating of perceived exertion (RPE). In general there was a greater magnitude of change in $\% \Delta PV$ in the cold condition than the temperate after 60 minutes of submaximal exercise, with a non-significant trend at the 90-minute mark as well. Additionally, $\% \Delta BM$ was lower for the cold environment condition, suggesting that the loss in plasma volume was due to a transient shift of water. It was also observed that HR, SBP, and RPE were greater during submaximal exercise than at rest.

These results suggest that while there is a large reduction in plasma volume during exercise in the cold, women are not experiencing negative effects due to dehydration. Instead, the subjects exhibited no difference in HR, SBP, DBP, and RPE between exercising conditions. Additionally, the subjects had lower $\% \Delta BM$ for the cold condition, indicating an increased retention of total body water as compared to the temperate condition. The evidence is consistent with what has been reported in the literature with regard to transient water shifts during submaximal exercise in the cold.

While this study identifies the presence of a resting water shift in the cold, it also has its limitations. Due to low sample size, significance across temperature conditions

was only found at one time point for $\% \Delta PV$ and trends for significance at additional time points. Future studies should include measuring pre and post urine specific gravity as an additional measure of hydration status, as well as a male control group for direct comparisons across temperature conditions, exercise duration, and exercise intensities. Additional studies in the area of cold induced resting water shifts should attempt to identify the temperature range at which this shift occurs and how the shift is affected by the luteal phase of the menstrual cycle.

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APPENDICES

APPENDIX A

INFORMED CONSENT DOCUMENT

SUBJECT CONSENT FORM FOR PARTICIPATION IN HUMAN RESEARCH
MONTANA STATE UNIVERSITY- BOZEMAN

PROJECT TITLE: The Effect of Exercising in the Cold on Markers of Fluid Balance in Women.

FUNDING: This study is NOT a funded project.

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PURPOSE: You have been invited to participate in a research study on the influence of cold exposure on fluid balance in female athletes. This study will help us better understand how markers of fluid balance change in response to exercising in cold and temperate environments.

Each participant is presented with this *Informed Consent Document* which explains the purpose of the testing and the associated benefits and risks of participation. It is the participant's responsibility to acquire medical clearance from her physician prior to lab testing. Each participant will also be screened by the student project director using responses provided by participants in a Health History Questionnaire. The screening process will only allow participants classified as "Low Risk", with no contraindications for testing, and no pre-existing conditions, to be in the study. This is in compliance with policies formulated by the American College of Sports Medicine¹.

PROJECT OUTLINE:

You (the participant) will report to the Movement Science Laboratory on three different testing sessions. At the first session, you will be asked to fill out a health history questionnaire. Session 1 will occur between days 5-7 of your menstrual cycle. Following completion of the questionnaire you will be asked to perform a graded exercise test on a cycle ergometer. For this test you'll be asked to wear a breathing mask that is connected to an open circuit spirometry measurement system. This will allow the researcher to measure your inflow of oxygen compared to your outflow of carbon dioxide throughout the test. For the test, you will cycle at a 70 rpm cadence, beginning at a 46 Watts low intensity, with workload increasing by 23 Watts every 4 minutes until lactate threshold is met. Upon meeting lactate threshold your work load will increase every minute until volitional exhaustion is achieved, or you are unable to maintain your pedaling cadence. The researcher will measure lactate levels using a lactate analyzer. Three

minutes into each interval a small drop of blood will be collected from your finger, which will be used to measure lactate. Heart rate will be monitored throughout this test. For descriptive data, measurements of body height and mass will also be collected.

Sessions 2 and 3 will occur either in the cold at the Montana State University Subzero Science and Engineering Research Facility (-5°C, 24°F) or in the temperate environment at the Montana State University Movement Science Laboratory (24°C, 73°F). You will be randomly assigned to either the cold environment or temperate environment. Session 2 will occur between days 7-10 of your menstrual cycle. Session 3 will occur one month later on the same day of your menstrual cycle.

On the cold experimental trial, you will wear athletic pants and long sleeved shirt, shoes, socks, hat, and gloves. You will be provided with the pants, long sleeved shirt, hat, and gloves. No cycling shorts may be worn for any of the testing sessions. Prior to cold exposure, nude body weight, a blood sample, resting heart rate and a urine sample will be taken and recorded. For nude body weight, you will stand on a scale inside a private room. The scale readout will be available outside of the room. The blood sample will be used to establish a baseline level of plasma volume. A small drop of blood will be collected from your finger and analyzed. Temperature in the cold chamber will be approximately -5° C, 24°F. You will exercise for 90 minutes at a power output of 90% of your lactate threshold. Breaks will occur for 1 minute at the 30 and 60 minute marks. Blood pressure, a fingerstick blood sample, heart rate and ratings of perceived exertion will be monitored and recorded every 30 minutes during the cold exposure. At the end of the 90 minutes, a final heart rate, blood pressure, blood sample, urine sample, and bodyweight will be recorded, after which you are free to leave. On the temperate experimental trial, you will wear a t-shirt, shorts, socks and shoes. The temperate trial follows the same protocol as the cold trial.

TIME COMMITMENT: The total time for your participation in this study is about 5 hours (about 1 hour for session 1, and 2 hours for sessions 2 and 3), distributed over two months. You are free to discontinue this study at any time.

RISKS: This study poses minimal risk to you. Some potential risks include abnormal blood pressure response to the cold, low heart rate, fatigue, and a sore finger from the fingerstick. You should be aware, that given the conditions, you may experience discomfort from the cold temperature. We will do our best to minimize risks, but keep in mind this study does not pose more risk than you encounter when you are outdoors during cold temperatures. If you perceive any warning signs (for example: numb hands, nauseousness or drowsiness, sudden change in heart rate) please notify the investigators. Wearing of the open circuit spirometry mask can feel uncomfortable. If you have any issues with claustrophobia or feel uncomfortable breathing, the mask will be taken off immediately. Additionally, during a maximal oxygen uptake test there is risk of muscle damage, high cardiovascular stress, and death. These risks are no greater than those experienced during moderate to high intensity activity. Screening prior to participation in the study will minimize these risks. If complications do arise during this study, we can refer you to a trained caregiver. However, there is no compensation available from MSU for injury. In order to participate in the study you will need to present proof of health insurance coverage.

SUBJECT COMPENSATION: You may receive a copy of your own test results. There are no other forms of compensation available for participating in this project.

BENEFITS: There are no direct benefits to you as a volunteer for this project. However, the Student Director, Kristen Croxford, is willing to discuss the interpretation of your own test results and overall study results upon completion of the project. You may contact Kristen Croxford by phone (541-331-8431) or by E-mail (kristen.croxford@msu.montana.edu) to discuss this option further.

CONFIDENTIALITY: The data and personal information will be regarded as privileged and confidential. Your test results will not be released to anyone besides the project directors except upon your written consent/request. Your right to privacy will be maintained in any ensuing analysis and/or presentation of the data by using coded identifications of each person's data.

FREEDOM OF CONSENT: You may withdraw consent for participation in writing, by telephone, or in person without prejudice or loss of benefits (as described above). *Participation in this project is completely voluntary.*

In the UNLIKELY event that your participation in this project results in physical injury to you, the Student Project Director will advise and assist you in receiving medical treatment. No compensation is available from Montana State University for injury, accidents, or expenses that may occur as a result of your participation in this project. Additionally, no compensation is available from Montana State University for injury, accidents, or expenses that may occur as a result of traveling to and from your appointments at the Movement Science / Human Performance Laboratory and the Montana State University Subzero Science and Engineering Research Facility. *Further information regarding medical treatment may be obtained by calling the Faculty Project Director, Dan Heil, at 406-994-6324, or the Student Project Director, Kristen Croxford, at 541-331-8431.* You are encouraged to express any questions, doubts, or concerns regarding this project. The Project Directors will attempt to answer all questions to the best of their ability prior to testing. The Project Directors fully intend to conduct the study with your best interest, safety, and comfort in mind. *Additional questions about the rights of human subjects can be answered by the Chairman of the Institutional Review Board, Mark Quinn, (406) 994-4707.*

The Effect of Exercising in the Cold on Markers of Fluid Balance in Women

Freedom of Consent

AUTHORIZATION: I have read the above and understand the discomforts, inconvenience and risk of this study. I, _____ (*name of subject*), agree to participate in this research. I understand that I may later refuse to participate, and that I may withdraw from the study at any time. I have received a copy of this consent form for my own records.

Signed: _____

Witness: _____ (optional)

Investigator: _____

Date: _____

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APPENDIX B

HEALTH HISTORY QUESTIONNAIRE

Health History Form

Personal Information

Name: _____ Sex: [] Male [] Female Date of Birth: ____ / ____ / ____ Age: _____
 Address: _____ City: _____ State: ____ Zip: _____
 Day Phone: (____) ____ - _____ Night Phone: (____) ____ - _____ Email: _____
 Height: _____ Weight: _____

Emergency Contact

Name: _____ Relationship: _____
 Day Phone: (____) ____ - _____ Night Phone: (____) ____ - _____
 Insurance: _____

Medications

List any prescribed medications you are currently taking: Reason

List any self-prescribed medications you are currently taking (including herbal and NSAIDS such as Advil, Motrin, Tylenol, etc.):

ACSM Coronary Artery Disease Risk Factors

To the best of your ability, please check the appropriate yes/no box for each of the following questions:

Risk Factor Defining Criteria Yes No

Family history:

Has your father or brother had a heart attack, stroke, or died suddenly of heart disease before the age of 55?

Has your mother or sister had a heart attack, stroke, or died suddenly of heart disease before the age of 65?

Cigarette Smoking Are you currently a cigarette smoker or have you quit within the past 6 months? _____

Hypertension (high blood pressure)

- Is your blood pressure over 140/90 mm Hg?
- Are you on medication to control your blood pressure?

Hypercholesterolemia (high cholesterol)

- Is your total serum cholesterol > 200 mg/dl, low-density lipoproteins (LDL) > 130 mg/dl, or high-density lipoproteins (HDL) < 35 mg/dl?
- Are you on medication to control your cholesterol?

Please list your cholesterol numbers if you know them:

Total: _____ LDL: _____ HDL: _____

Impaired fasting glucose Do you have diabetes mellitus?

Have you had fasting blood glucose measurements of ≥ 110 mg/dL confirmed on at least 2 separate occasions?

Sedentary lifestyle Are you physically inactive and/or sedentary (little physical exercise on the job or after work)?

Do you have any of the following known diseases? Please elaborate on any yes answers below.

Category Diseases Yes No

Cardiovascular Cardiac, peripheral vascular, or cerebrovascular disease _____

Pulmonary Chronic obstructive pulmonary disease, asthma, interstitial lung disease, cystic fibrosis

Metabolic Diabetes mellitus (type I or II), thyroid disorders, renal or liver disease _____

Comments:

Signs and Symptoms. Please elaborate on any yes answers below.

Yes No

Have you experienced unusual pain or discomfort in your chest (pain due to blockage in coronary arteries of the heart)?

Have you experienced unusual shortness of breath during moderate exercise (such as climbing stairs)? _____

Have you had any problems with dizziness or fainting? _____

When you stand up, or sometimes during the night, do you have difficulty breathing? _____

Do you suffer from swelling of the ankles (ankle edema)? _____

Have you experienced a rapid throbbing or fluttering of the heart? _____

Have you experienced severe pain in your leg muscles during walking? _____

Has your doctor told you that you have a heart murmur? _____

Have you felt unusual fatigue or shortness of breath with usual activities? _____

Comments:

Musculoskeletal

Yes No

Do you have any current musculoskeletal limitations that would impair your ability to perform maximal exercise (back pain; swollen, stiff, or painful joints; arthritis; etc.)? If yes, please explain below.

Comments:

Other

Please list and explain any other significant medical problems that you consider important for us to know:

Training History and Goals

Your answers to the following questions will help us determine the most appropriate protocol to use during your VO₂MAX test.

EXERCISE

Are you currently involved in a regular training program? Yes No

Frequency (x / wk) Duration (minutes, miles, etc / session) Type of exercise

Cardiovascular _____

Strength training _____

Flexibility _____

Assess your overall fitness in each of the categories:

Cardiovascular Excellent Good Fair Poor Don.t know

Strength Excellent Good Fair Poor Don.t know

Flexibility Excellent Good Fair Poor Don.t know