RESTING HEMODYNAMIC FUNCTION AND REACTIVITY TO ACUTE STRESS:
THE INFLUENCE OF HYDRATION ON CARDIAC FUNCTION
AND PLASMA VOLUME

A thesis presented to the faculty of
the College of Arts and Sciences of Ohio University

In partial fulfillment of the requirements
for the degree
Master of Science

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November 2004
This thesis entitled
RESTING HEMODYNAMIC FUNCTION AND REACTIVITY TO ACUTE STRESS:
THE INFLUENCE OF HYDRATION ON CARDIAC FUNCTION
AND PLASMA VOLUME

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The purpose of this study was to assess the effects of hydration status on cardiac function and hematological factors at rest and during psychological stress. Volunteers participated in an initial visit (Session 1), a pre fluid-load visit (Session 2), and a post fluid-load/stress manipulation visit (Session 3). At each session, blood pressure (SBP, DBP), heart rate (HR), and total body water (TBW) were obtained. At Session 2, participants were assigned to either an Enhanced Hydration (EnH) or Non-Enhanced (NE) condition. The EnH Group drank 6 liters of water over 3 days proceeding Session 3. During Session 3, cardiac and hematological measures were recorded during a seated baseline, math task, and a cold pressor task. Session 1 analyses revealed significant inverse relationships for males between DBP and TBW. At session 3, participants in the EnH Group displayed greater HR at rest compared to the NE Group. Stress-reactivity analyses for the math task revealed significant Group differences on DBP with the NE Group displaying greater DBP reactivity compared to the EnH Group. These results indicate differential effects of hydration status on cardiac function while at rest and during psychological stress.

Approved:

Stephen M. Patterson

Professor of Psychology
Acknowledgments

My advisor, Steve Patterson, has become an invaluable person in my career. I would graciously like to thank him for his guidance throughout each stage of my thesis project. Steve was a great source of inspiration and has accentuated my interest in health psychology. I am very fortunate to have received the amount of personal attention and support he has bestowed upon me.

In addition, I would like to thank my other committee members Chris France and Dan Lassiter who provided thoughtful suggestions in improving this project. Chris was especially helpful in answering impromptu questions and providing expert advice.

On a personal note, I would like to thank Jennifer Ratcliff for her friendship during this process. Her informal feedback and compassion was greatly appreciated. I would also like to thank Dan Prause for his love and warmheartedness throughout this project. He offered me encouragement and continually emphasized my ability to succeed. His understanding and support will always be remembered.
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Introduction

Of all the nutrients required by the human body, water is the most essential, yet most often overlooked, daily requirement for proper physiological functioning and survival (Berdanier, 2000). Proper hydration is necessary for the body to sustain normal physiological functioning such as digestion or temperature regulation. Furthermore, water is the most abundant element found in the body, as the adult human body consists of approximately 60% water. However, with daily hydration losses equaling about 4% of body mass, it is possible for individuals to not replenish all the water lost on a given day and therefore be at a hydration deficit chronically (Wildman & Medeiros, 2000). In fact, much of society in the United States and elsewhere are believed to be mildly dehydrated. The American Dietetic Association asserts that people should imbibe 1.8-2.8 liters of water daily (Duyff, 1998). Despite this fact, the 1977-1978 Nationwide Food Consumption Survey of 17,062 adults found the median water intake amount to be only 662 milliliters for adults (Enns, Goldman, & Cook, 1997; Food and Nutrition Board, 1989). A more recent survey conducted by Yankelovich Partners for the Nutrition Information Center and the International Bottled Water Association found on average people consume only 1,088 milliliters of water (Grattan, 1998). Although this recent estimate indicates that water consumption has increased over the past 25 years, total water intake still falls short of the recommended daily amount. This less than optimal level of hydration can cause physical discomfort, impaired physical and mental performance, and result in acute and chronic health consequences. Mild dehydration can cause gastrointestinal discomfort, appetite loss, and cramping, while more severe
dehydration can contribute to a multitude of physical ailments and eventually lead to death (Felesky-Hunt, 2001).

Despite the importance of hydration, most studies in the behavioral health sciences have not assessed hydration and its influence on resting physiological functioning, psychophysiological reactivity during psychological and physical stress, nor its impact on behavioral medicine research methodology and study design. A number of studies have investigated the effect of acute hydration on several physiological processes such as renal function (Light & Turner, 1992; Light, Koepke, Obrist, Willis, 1983), changes in plasma volume and hemoconcentration (increased ratio of cellular constituents to blood plasma; Patterson, VanderKaay, & Arnott, 2001), and cardiovascular reactivity (VanderKaay, Patterson, & Shan Holtzer, 2002; Patterson, France, Prause, & Gill, 2002). These studies have illustrated that acutely increasing fluid in the body through consumption of fluid preceding physical and psychological stressors influences physiological responses during stress. However, these studies only investigated the effects of acute increases in hydration and therefore were unable to assess whether long-term hydration has similar effects on stress responding.

To the knowledge of this investigator, only four studies have been conducted to assess the effect of long-term hydration enhancement on physiological functioning (Kristal-Boneh, Glusman, Shitrit, Chaemovitz, & Cassuto, 1995; Shore et al., 1988; Patterson & Spinks, 2002; Prause, Patterson, France, & Spinks, 2002). However, for three of these studies investigating the effects of hydration on cardiac function was not the main goal, and the fourth study did examine cardiovascular reactivity, but was limited
by the use of a weak stressor which produced small physiological change. In short, no study has been designed to specifically assess the effect of long-term hydration enhancement on stress induced hemoconcentration and cardiovascular reactivity using a potent psychological stressor. Therefore the current investigation examined this relationship.

The primary goal of the present study was to investigate the effect of an enhanced hydration regimen on cardiac function (resting and reactivity to acute stress). This was accomplished by examining group differences (Enhanced Hydration Group vs. Non-Enhanced Group) in cardiovascular functioning and reactivity using impedance cardiography. This study also assessed the influence of hydration enhancement on resting plasma volume and stress-induced hemoconcentration. In addition, the relationship between changes in total body water and resting blood pressure and plasma volume was examined. Finally, this study explored the effect of long-term hydration enhancement on 24-hour blood pressure and nocturnal blood pressure decline during sleep using ambulatory blood pressure monitoring.

The following literature review spans and integrates several areas of research. It begins with a further discussion of the benefits of being fully hydrated as well as the negative effects of dehydration. Although a great depth of information on dehydration exists, review of this area was limited to research that assessed the general effects of dehydration on performance, the body, or health. This is followed by an appraisal of psychological stress research that has specifically investigated the effects of stress on hemoconcentration and the effect of hydration on stress responding. Finally, the overall
The purpose of the current investigation is presented as well as the specific aims and hypotheses.

**Role of Proper Hydration on Health**

The human body primarily consists of water, which is responsible for carrying out many life-sustaining processes. Proper hydration allows oxygen and nutrients to move freely throughout the body. Water aids in the digestion and absorption of food by serving as a solvent for nutrients as well as being the medium through which toxins and waste products are removed (Kleiner, 1999). Water is a key component for regulating body temperature; it cools the body through evaporation of water left on the skin from perspiration (Whitmire, 1996). Water is also beneficial to the cardiovascular system and other organs because it serves as a lubricant for the body. When water combines with other nutrients such as protein it protects the heart, intestines, eyes, lungs and joints by acting as a lubricant as well as when it is secreted from salivary glands, respiratory system and the gastrointestinal track (Berdanier, 2000). Therefore, without doubt, water is crucial for proper body functioning.

**Body Water Distribution and Balance**

Water is contained in and is an essential part of all tissues in the body. Depending on the amount of body fat, 50 to 80% of body weight is accounted for by water and this quantity of water is usually referred to as total body water (Food and Nutrition Board, 1989). Total body water consists of two general fluid compartments in the body: intracellular water and extracellular water compartments. As its name describes, intracellular water is literally the water contained within all of the cells of the body.
Intracellular water accounts for 55% of total body water or approximately 35-45% of body weight (Berdanier, 2000; Berne & Levy, 1998; Pierson, Wang, Thornton, & Heymsfield, 1998). Extracellular water is water found outside the cells and comprises primarily interstitial fluid, plasma and lymph, making up approximately 45% of total body water or about 20-30% of body weight (Berdanier; Berne & Levy; Pierson et al.).

In a healthy adult, there is a daily balance between net water gain from food and fluid consumption and loss via diuresis, respiration, and sweat, which allows the percentage of total body water to remain fairly stable. Adolph (1943) reported that under normal conditions of proper hydration and fluid loss, daily total body water can fluctuate as little as ±0.22% of body weight. This continual state of normal body water is referred to as euhydration in which the amount of daily water intake equals the amount of daily water output. During fluid loss, body water is lost through urine, lungs, fecal water, and thermal regulation (sweat) which collectively account for 2,500 to 3,000 milliliters of daily water loss (Greenleaf, 1982a; Kleiner, 1999). Conversely, fluid intake, food moisture, and oxidation within body tissues compensate for this deficit (Greenleaf, 1982a). Although food moisture and cellular oxidation contributes approximately 1,000 milliliters and 250 milliliters respectively, the majority of body water lost must be replaced through fluid consumption (Kleiner).

**Daily Fluid Intake Recommendations**

Given that body water must primarily be replenished through fluid intake, the Food and Nutrition Board (1989) recommends that the typical adult under average conditions needs to consume 1 milliliter of water for every kcal of energy burned.
However, the actual amount of fluid intake will depend on physical activity and environmental conditions. In fact, the Food and Nutrition Board promoted increasing the water requirement to 1.5 milliliters/kcal to allow for variations in physical activity. Therefore, the average male should consume 2,900 milliliters of water daily and the average female should consume 2,200 milliliters of water each day (Kleiner, 1999). In addition to the standard recommendation of fluid intake, the American Dietetic Association advocates increasing water consumption for specific circumstances (Duyff, 1998). For instance, people should increase their fluid ingestion when exposed to extreme hot or cold, recirculated air, and strenuous work or exercise. Latzka and Montain (1999) further assert that depending on the extremeness of the environment or physical activity, fluid needs may range from 2-16 liters per day. Fluid intake should also be increased during times of sickness, pregnancy or breast-feeding, and when eating a fibrous diet (Duyff). Furthermore, Maughan and Nadel (2000) report that although the kidneys are responsible for excretion and regulation of body water, they only operate efficiently when fluid consumption exceeds necessary requirements. Hence, although there are established guidelines for fluid consumption, people should imbibe more than the minimal fluid requirement for the body to operate efficiently under varying activities and conditions.

**Health Consequences of Dehydration**

The established fluid guidelines recommend the average fluid intake required to keep the majority of people in society adequately hydrated. Naturally, if people do not meet the proper daily fluid intake to maintain good health, a deficit in hydration status would result. Greenleaf (1982a, 1992) has investigated the effects of hydration on the
human body and has subsequently identified typical consequences that occur with dehydration. Greenleaf reported the observed negative consequences during percent losses of body weight. As little as 0.5% loss of body weight will cause a person to experience thirst, whereas a 2% loss leads to intense thirst, nebulous discomfort, and decreased appetite. Vascular compartment hemoconcentration (increased ratio of cellular constituents to plasma), decreased urine output, and dry mouth result at a 3% loss. Hydration losses of 4-5% of body weight lead to a variety of symptoms. Specifically, Greenleaf reported a person could experience impatience, sleepiness, nausea, headaches, difficulties concentrating, and emotional instability. Greater decreases in hydration lead to increasingly more severe consequences. At an 11% loss of body weight there is extreme hemoconcentration and decreased blood volume that results in failure of the blood to circulate normally and eventually dehydration could result in death (Felesky-Hunt, 2001). Given all the negative consequences of acute dehydration, even hypohydration can have a negative impact on long-term health.

Mild to moderate dehydration occurs with minimal loss of body water and has been defined as a 1 to 2% decrease in body weight due to fluid loss (Felesky-Hunt, 2001; Barr, 1999). This state of dehydration, or hypohydration, has been associated with several disease states. In Particular, Michaud et al. (1999) investigated the relationship between fluid intake and the risk of bladder cancer in men. This 10-year correlational study found that total daily fluid intake is negatively related to bladder cancer risk even after adjusting for other potential risk factors, such as age, geographic region, smoking status, number of packs smoked per year, energy intake, and fruit and vegetable intake. When analyzed by
type of beverage, Michaud et al. discovered that only water intake had this significant negative relationship. As a result, the authors concluded that high water intake is related to a decreased bladder cancer risk. Comparatively, other studies have found similar negative effects between water intake and disease. Shannon, White, Shattuck, and Potter (1996) found a negative association between water intake and the risk of colon cancer for women. A marginal relationship between increased water consumption and decreased colon cancer risk was also detected in men. In addition, Potter, Slattery, Bostick, and Gapstur (1993) reported a negative association between colon cancer risk and intake of vegetables, which consist of 85-95% water (Felesky-Hunt, 2001). A 6-year correlational study assessed the relationship between water intake and fatal coronary heart disease (Chan, Knutsen, Blix, Lee, & Fraser, 2002). This study found an inverse relationship between the amount of water consumed daily and fatal incidents of heart disease. A positive relationship between the intake of other beverages and fatal heart disease was also found. Borghi et al. (1996) ascertained that a treatment of high water intake for patients with urinary tract stones resulted in a greater percentage of stone free patients 5 years later when compared to a control group without the enhanced hydration regimen. Kurabayashi, Kubota, Tamura, and Shirakura (1991) found that water consumption at night reduced the viscosity of blood by morning and consequently proposed that a glass of water at night could prevent morning occurrences of cerebral infarction (obstructive stroke). In addition, Stookey (1999) asserted that a diet low in water content appears to be related to chronic diseases, such as obesity, diabetes, cardiovascular disease, hypertension, and cancer.
Given that body water is the most abundant constituent of the human body, it is not surprising that dehydration appears to be linked to chronic disease. Yet, hydration status is often overlooked in studies investigating chronic illness. Furthermore, as Lax, Eicher, and Goldberg (1992) point out, hydration status could lead to misdiagnosis on medical tests. Their research discovered that mild dehydration could produce mitral valve prolapse in women who did not have the condition in a euhydrated state. Although this observation was not found in men, women typically have mitral valve prolapse more than men and therefore may be more susceptible to this condition in a dehydrated state (Aufderheide, Lax, & Goldberg, 1994). Despite the fact hypohydration might be connected to disease and produce misleading results on medical tests, the variability in hydration status is essentially overlooked in studies conducted by researchers in behavioral medicine and health psychology. Consequently, this oversight could lead to inaccurate research and medical test results if the participants are dehydrated or if society’s variability in hydration status is not accounted for in research.

In summary, dehydration produces a multitude of acute and long-term health consequences. Dehydration causes acute episodes of vague discomfort, hemoconcentration, difficulty concentrating, and extreme dehydration could even lead to death. The effects of long-term mild dehydration on health are increased bladder and colon cancer risk, fatal coronary heart disease, and possibly hypertension. Hydration status may influence medical test results, which could produce inconsistencies in diagnosis. Moreover, enhanced hydration is shown to have positive benefits, such as absence of urinary tract stones and reduced blood viscosity which could help alleviate
cerebral infarction. In other words, although not definite, there is suggestive evidence for hypohydration adversely affecting health and possibly being a key factor in the etiology of several diseases.

Effects of Acute Dehydration on Physical and Mental Performance

In addition to the long-term health effects of chronic mild dehydration, insufficient hydration can also lead to inadequate physical performance and cognitive impairment. Research has demonstrated that hydration deficits of 2% result in significant cognitive impairments (Gopinathan, Pichan, & Sharma, 1988). This study conducted by Gopinathan at el. showed deterioration in short-term memory, mathematical performance, and, as indicated by an army trail-marking test, motor speed and attention. Cian, Barraud, Melin, and Raphel (2001) found similar performance consequences after 30 minutes (test 1) in their repeated measures design consisting of dehydrated, control, and hydrated sessions. Although after 3.5 hours (test 2) they observed no differences in short-term memory impairment between the dehydrated and control sessions (improvements in the dehydrated group may possibly be due to both groups receiving 100 milliliter of glucose water prior to test 2 and the control participants having a drop in hydration status equal to a 0.78% drop in body weight), the hydrated session showed improvement in long-term memory compared to the control or dehydrated sessions. In general, these studies point to dehydration having negative effects on cognitive functioning.

In addition to the insufficiencies in mental performance, dehydration also adversely affects exercise performance and temperature regulation of the body. For example, Sawka (1992) reported that during hypohydration (a state of reduced total body
water) there was a diminished physical work capacity and diminished maximum aerobic power, as well as elevated core temperature during exercise. Dehydration need not be great to negatively affect performance. A water loss as little as 2% of body weight can affect the body by decreasing aerobic power and impairing exercise performance (Latzka & Montain, 1999). Dehydration during exercise results in reduced stroke volume (amount of blood pumped per beat) and increased heart rate (González-Alonso, 1998).

Furthermore, Latzka et al. (1998) found that when participants exercised under heat stress during a hyperhydration (an enhanced state of total body water) session heart rate was lower compared to a control (euhydrated) session, therefore suggesting that enhanced hydration may have positive performance effects and dehydration results in less than optimal performance.

In sum, the abovementioned studies demonstrate that dehydration can decrease both physical and mental performance. Thus, insufficient hydration not only affects physical health as previously stated, but also appears to produce deficits in both physical and cognitive performance. Therefore, the assessment of hydration status is relevant for physiological research, but as yet is a largely unexplored area.

**Daily Water Drinking Behaviors**

Despite the recommendations of daily water intake and the potential negative health effects of long-term dehydration, people in the United States and other countries tend not to drink enough water. In a survey of water consumption by individuals living in the Netherlands, United Kingdom, and Canada, daily water consumption in 1978 was found to be roughly 1,300 milliliters per person (Zoeteman, 1985). A more recent survey
designed to estimate water intake in Canada revealed an individual fluid consumption of approximately 1,620 milliliters per day (Levallois et al., 1998). One strength of this survey was that it included diet diary recordings of actual water ingestion as well as water from other beverages and food. This generally would give a more accurate estimate of fluid intake because food moisture contributes up to 1 liter of water per day. However, due to a low response rate of 14% (n = 139), this study’s estimate may not be representative of the Canadian population. Overall, when comparing the fluid consumption in these countries to U.S. recommended dietary allowances, water intake appears to fall below recommended daily fluid intake.

As with other countries, published data on hydration status or water intake in the United States is limited. Reports on fluid consumption are mostly derived from data collected by the U.S. Department of Agriculture, which has conducted several national surveys: the 1977-1978 Nationwide Food Consumption Survey (NFCS) that assessed 17,062 adults, the 1989-1991 Continuing Survey of Food Intakes by Individuals (CSFII) that assessed 10,448 adults, and the 1994-1996 CSFII that assessed 6,636 adults (Enns et al., 1997). Each of these surveys collected data on U.S. food and beverage consumption. The 1977-1978 NFCS and 1989-1991 CSFII used a 1-day diet recall and a 2-day diet diary to collect data for 3 consecutive days; the 1994-1996 CSFII used a 1-day diet recall to collect data for 2 non-consecutive days (Enns et al.).

Using data from the 1977-1978 NFCS and the 1994-1996 CSFII, Heller, Sohn, Burt, and Eklund (1999) reported a trend increase in water consumption between the two surveys for people age 20 to 64. However, the differences in fluid intake between the two
surveys were small (roughly 10 to 11 milliliters per kilogram of body weight for drinking water and 32.5 to 36.5 milliliters per kilogram for total fluid regardless of water content) and the differences in water consumption were not tested statistically. Using data from the 1994-1996 CSFII, Grandjean, Reimer, Bannick, and Haven (2000) state that the fluid intake for males (age 20-39) is about 1,441 milliliters, which falls well below recommended levels. Kleiner (1999) estimated from the 1977-1978 NFCS the average daily water intake to be 1,128 milliliters for non-lactating females. This approximation included water added to make beverages such as juice or coffee. Yet, Ershow, Brown, and Cantor (1991) reported from the 1977-1978 NFCS total water consumption (food + fluid) to be 1,940 milliliters for all females. This again falls below the recommended fluid intake. The difference in water consumption estimates between the Ershow et al. study and Kleiner’s estimation is most likely due to the latter not including the water content of food. However, using this data as an estimate for the entire population, Kleiner concluded that much of society may suffer from chronic mild dehydration.

In the aforementioned studies on national water ingestion behaviors, it is important to note that even when water added to food and beverages was included, the estimates of water consumption fell below recommended guidelines. One contributing factor to this less than optimal fluid intake could be involuntary dehydration which can occur due to the fact that people stop experiencing thirst before they have completely replenished water lost to dehydration (Ramsay, 1989; Rolls, Wood, Rolls, Lind, & Ledingham, 1980). In fact, Greenleaf (1982b) reported that participants, after showering and eating following exercise in the heat, were no longer thirsty and felt recovered even
though they were still dehydrated by 4-5 liters of water. Hence, these researchers illustrate that people are ineffectual at determining whether they are hydrated during episodes of dehydration, thus implicating the importance of a daily hydration regimen. Lacking a daily routine of drinking the recommended amounts of water may more easily lead to the state of involuntary dehydration.

In conclusion, the average recommended water intake ranges from 2.2 liters (for females) to 2.9 liters (for males) of water daily (Kleiner, 1999). Nevertheless, published research in the United States and other countries demonstrate that society does not drink the recommend amount of water. A plausible explanation for society’s tendency towards mild dehydration is that people are ineffectual at determining their own state of dehydration and thus do not adequately replenish body water.

Psychological Stress

Although hydration is an indispensable aspect of maintaining proper health and dehydration has adverse effects on the body, the potential impact of hydration status on the physiological effects of psychological stress on hydration behavior have remained relatively untapped in behavioral medicine and health psychology research and methodology. Until recently there was no reason to suspect hydration status as a potential mediating factor linking psychological stress and disease development, even though the relationship between stress and disease has been studied for decades. Cannon (1914, 1929) was among the first researchers to explore possible connections between stress and diseases by studying the physiological effects of stress in animals. He described the “fight or flight” response whereby an organism responds to threat or danger with heightened
sympathetic nervous system activity which increases heart rate, blood glucose, sweating, the redistribution of blood from the gastrointestinal track to the muscles, and heightens blood coagulation to assist during injury. Cannon believed psychological stress could cause health problems because of the physiological response to stress. He found emotional stress in cats increased heart rate, blood pressure, and the concentration of red blood cells circulating in the vascular system (hemoconcentration) which over time could cause organ damage. Selye (1936, 1950) found similar hemoconcentration effects during stress while researching and theorizing his General Adaptation Syndrome. This theory of stress responding states that with long-term exposure to stress the body would be physiologically unable to resist unrelenting stress, resulting in immune system breakdown, illness, disease, and possibly death.

Since this early research, physiological responsiveness to stress and the relationship to cardiovascular diseases (particularly coronary heart disease and hypertension) has become a widely studied area in behavioral medicine (Krantz & Manuck, 1984). Coronary heart disease results from a build up of plaques (atherosclerosis) and hardening of the arterial walls, which can result in ischemia (obstruction of blood flow), myocardial infarction (heart attack), and stroke. Krantz and Manuck reported there are several mechanisms which could cause the initial injury leading to atherosclerosis and the prevention of healing, which are related to hemodynamic factors, endocrine substances, blood lipids, or immunologic factors. They assert many of the disruptions of normal physiological processes that are implicated in disease states are also observed in psychological stress research. Although research has
shown a relationship between stress and cardiovascular disease, the underlying physiological link has remained relatively elusive. One possible factor that links stress and heart disease, which is also directly related to hydration status, is plasma volume and stress-hemoconcentration. Due in part to this connection, several researchers have in recent years begun studying the effects of stress on plasma volume and the subsequent hemoconcentration of cells, lipids, and proteins in the blood stream.

**Stress and Hemoconcentration**

Psychological stress is shown to elicit increases in the amount of lipids circulating in blood (Dimsdale & Herd, 1982; Stoney, Matthews, McDonald, & Johnson, 1988; Mattiasson, Lindgärde, Nilsson, & Theorell, 1990). One of the proposed mechanisms behind this stress-induced augmentation in cholesterol is hemoconcentration. Stress-induced hemoconcentration signifies hemoconcentration that is caused by fluid movement from within the vascular compartment to the interstitial space. Several researchers investigated whether this type of hemoconcentration played a role in the altered lipid levels observed during stress.

Muldoon et al. (1992) explored this relationship in 26 healthy males who participated in three tasks: Stroop color-word interference test (10-minute), mental arithmetic (10-minute), and posture manipulation (20-minute). Measurements of systolic blood pressure, diastolic blood pressure, total serum cholesterol, hematocrit (percentage of blood volume occupied by cells) and hemoglobin (respiratory protein of red blood cells) were obtained during baseline, task, and recovery periods. Hematocrit and hemoglobin was used to calculate plasma volume. Results revealed that although
increased total cholesterol was seen during all three tasks, after accounting for the effects of hemoconcentration (i.e. decreased plasma volume) the changes in cholesterol were no longer significant. Patterson, Grottiener, Hecht, Vargot, and Krantz (1993) found similar serum lipid results in a sample of 23 healthy male volunteers who underwent a 10-minute mental arithmetic task with mild verbal harassment. Likewise, Patterson, Matthews, Allen, and Owens (1995) tested 17 healthy female participants during the follicular phase of their menstrual cycle and found increased hemoconcentration during a 3-minute speech task stressor. All these studies (Muldoon et al.; Patterson et al., 1993; Patterson, Matthews, et al., 1995) observed significant increased systolic blood pressure and diastolic blood pressure during mental stress and increased hemoconcentration evidenced by hematocrit and hemoglobin calculations of plasma volume.

In a recent study, Bachen, Muldoon, Matthews, and Manuck (2002) demonstrated comparable results. They randomly assigned 52 healthy male participants to either a labetalol (attenuated sympathetic activation evidenced by reduced heart rate reactivity but did not attenuate blood pressure) group or saline group and either a stress (modified Stroop color-word inference test (8-minute), mental arithmetic (5-minute), and public speaking (5-minute)) or no-stress group. Consistent with previous research they found that stress induced changes in systolic blood pressure, diastolic blood pressure, and hemoconcentration. In addition, serum lipids were not significantly affected by the labetalol which again suggested hemoconcentration was responsible for the augmented lipid profiles.
In contrast to Muldoon et al. (1992) who did not observe plasma volume returning to baseline during recovery after psychological stress, Patterson et al. (1993) did observe calculated plasma volume levels during recovery that were not significantly different from baseline. The rapid return of plasma volume following a stressor has since been repeated in a sample comprising both male and female participants. Specifically, Patterson, Krantz, and Jochum (1995) found plasma volume returned to baseline levels 12 minutes after a 10-minute arithmetic task with mild verbal harassment. The researchers of these abovementioned studies on hemoconcentration proposed that the likely mechanism for this stress-induced hemoconcentration is the increased blood pressure observed during stress.

In sum, these studies illustrate several important details. First, different durations of physical stress and several types of psychological stress consistently induce stress-hemoconcentration effects. Second, unlike exercise-induced hemoconcentration, which is mainly caused by fluid loss, stress hemoconcentration results from the redistribution of fluid (plasma volume) during an acute stress task. This is evidenced by the rapid return of plasma volume following the cessation of a stressor. Third, stress induced changes in hemoconcentration can reliably be detected through changes in plasma volume calculated from hematocrit and hemoglobin. Finally, stress induces cardiovascular reactivity indicated by increased systolic blood pressure and diastolic blood pressure; a rise in blood pressure is thought to be the driving force behind these stress-induced changes in hemoconcentration.
Nevertheless, these studies also illustrate several shortcomings and implications for continued investigation in this area of research. Even though cardiovascular reactivity is not the main focus of these studies, a more dynamic measure of reactivity could be used. For example, blood pressure, which is defined as pressure within the vascular system expressed in millimeters of mercury (mmHg), is actually determined by the amount of blood pumped per minute (cardiac output) and the amount of resistance the vascular system places on the flow of blood (total peripheral resistance). Using these indices in addition to blood pressure and heart rate would give a better indication of the effects of stress on cardiovascular reactivity and hemoconcentration. Furthermore, none of the aforementioned studies reported that the participants refrained from all beverages (including water). By not asking participants to refrain from water, participants will vary on the amount of water contained in the vascular system. Turner (1994) reports that 5 liters of water is contained in the total amount of blood volume and that blood volume partially determines blood pressure. Thus, participants could differ on resting and reactivity blood pressure measurements simply due to differences in water consumption. Because this research area examines stress-induced fluid shifts and indicates blood pressure as a possible mechanism behind stress-hemoconcentration, an oversight in this research is not investigating or controlling for the effects of fluid hydration.

In a novel contribution to this area, Patterson, VanderKaay, Shanholtzer, and Tulodzieski (2002) were the first to investigate the effects of hydration on serum lipid levels at rest and during psychological stress. Plasma volume and cholesterol were recorded during baseline and a math task in 13 individuals who were tested twice: once in
a hydrated state (12 hours without food or fluid plus fluid loading prior to testing), and once in a hypohydrated state (12 hours without food or fluid). Results showed higher lipid levels during baseline and math during the hypohydrated state. Further, there were greater changes in hemoconcentration and lipids when participants were hydrated compared to the hypohydrated state. As these results demonstrate, hydration influences both resting and reactivity measurements of lipid profiles, which further exemplifies the importance of assessing individual hydration status in research that is designed to examine biological substances that are subject to hemoconcentration effects.

Accordingly, this study illustrates an important methodological implication for this research area. The aforementioned lipid studies demonstrated that stress-hemoconcentration leads to increased concentration of blood particles during acute stress. The Patterson, VanderKaay, et al. (2002) study demonstrated that variability in hydration status affects physiological measurements obtained at rest and during psychological stress. Therefore, it is possible that a person’s hydration status could influence a multitude of physiological measures examined in behavioral medicine research.

**Acute Hydration during Stress**

Several studies explored the stress-hydration relationship using an acute fluid loading protocol in psychophysiological research. Although not investigating the effects of hydration per se, Light et al. (1983) examined sodium and fluid excretion in 40 male participants during a 5-hour protocol (hours 1 and 2 seated rest, hour 3 baseline, hour 4 stress, hour 5 recovery). Participants were assigned to either a stress (60-minute competitive task with monetary incentives) or non-stress condition. Fluid loading was
achieved by having participants imbibe 1 liter of water during hour 1 and 200 additional milliliters every 30 minutes during hours 2 through 5. Urine voids were obtained every 60 minutes and stabilized sodium and fluid excretion were attained by hour 3. Over hours 1 through 3 blood pressure remained consistent and heart rate decreased slightly. Results revealed that only participants with a high risk for hypertension and high heart rate reactivity showed sodium and fluid reductions during stress; the other participants demonstrated no reliable changes. For the high risk group, greater heart rate increases during stress was related to decreased sodium and fluid excretion and therefore it is inferred that increased hydration in this group produces elevated heart rate.

A similar protocol investigated sodium excretion in 28 normotensive males (Light and Turner, 1992). The main difference from the Light et al. (1983) study was that these participants underwent a controlled diet and 24-hour urine collections for 3 days preceding the laboratory protocol. In addition, during hour 1 they consumed a standardized meal along with the water. Throughout the remaining protocol urine voids and a 200 milliliter fluid consumption was required every 30 minutes. Unlike the previous study, cardiovascular reactivity was obtained using impedance cardiography measurements of heart rate, cardiac output, and total peripheral resistance. The majority (71%) of men exhibited a faster sodium loss during stress compared to baseline (fast natriuresis) whereas the remaining men exhibited a slower sodium loss (slow natriuresis); however the baseline and final recovery sodium excretion amounts did not significantly differ. At baseline slow natriuresis participants had higher systolic blood pressure,
diastolic blood pressure, and marginally higher total peripheral resistance; greater heart rate and cardiac output reactivity was demonstrated during stress.

Although these researchers use an intensive fluid loading regimen primarily to investigate renal functioning, not hydration status and hemodynamic reactivity, they did demonstrate that during extreme hyperhydration conditions, differences in sodium and fluid output can be observed in both normotensive and high risk groups. Unfortunately, this study did not take advantage of the hyperhydration status of its participants and examine the possible associations between hydration status and blood pressure. Another shortcoming of these two studies is the acute nature and extremeness of the hydration regimen. Due to the high volume of fluid ingested in a relatively short time, results may not be representative of typical fluid and sodium excretion occurring during stress in a euhydrated state. Moreover, hydration status in these studies was inferred, because the goal of these studies was not to look at hydration status, but instead kidney function during stress. Thus, this study did not assess the effects of hydration on cardiovascular reactivity.

Using a less extreme acute fluid loading protocol, a few researchers have specifically examined the effects of hydration on cardiovascular reactivity and hemoconcentration during stress. Patterson et al. (2001) were the first to explore this phenomenon in 13 healthy male and female volunteers who performed a 6-minute mental arithmetic task as cardiovascular and hematological measures were collected. After abstaining from food, beverages, and exercise for 12 hour prior to testing, participants were randomly assigned to either a hydrated (fluid loaded) or non-hydrated (no fluid)
condition. Hydrated participants ingested 12 milliliters per kilogram of body weight of a commercially available rehydration beverage preceding a 10-minute baseline period. Automated blood pressure and impedance cardiography recordings of cardiac output and total peripheral resistance were obtained during the baseline, math task and recovery periods. Collection of blood samples for the assessment of plasma volume also occurred during baseline, task, and recovery periods. Results revealed acute fluid loading did have an overall effect on plasma volume (evidenced by higher plasma volume at rest and during stress); however, hydration did not attenuate stress hemoconcentration. On the other hand, hydrated participants had greater cardiac output and lower total peripheral resistance at rest in addition to greater cardiac output reactivity during stress. Hence, fluid loading was found to affect cardiovascular reactivity.

Likewise, VanderKaay, Patterson, and Shanholtzer (2002) found similar effects of hydration on hemodynamic reactivity. Using a repeated measures design, they examined the influence of an acute hydration enhancement (Gatorade or Water) or no hydration enhancement on stress hemoconcentration and cardiovascular reactivity. Fifty male and female college students participated in a laboratory protocol similar to the Patterson et al. (2001) study, except these participants underwent mental arithmetic with mild verbal harassment. Blood and cardiovascular measurements were also obtained in the same manner as the Patterson et al. study (2001). In addition, bioelectrical impedance measurements of intracellular water, extracellular water, and total body water were obtained at the beginning of each visit. Results demonstrated that during the hydration enhancement session participants had an overall increase in plasma volume, cardiac
output, and systolic blood pressure compared to the non-hydrated session. Furthermore, the hydration enhancement condition produced increased reactivity during stress (evidenced by greater decreases in total peripheral resistance and heightened cardiac output) as compared to the non-enhancement condition. Thus, acute hydration enhancement resulted in decreased constriction of the arterioles and an increase in the amount of blood pumped per minute by the heart during stress.

A second goal of the VanderKaay, Patterson, and Shanholtzer (2002) study was to investigate the relationship between a participant’s initial hydration status, as assessed by bioelectrical impedance, and cardiovascular reactivity to stress. Therefore, in order to assess this relationship, only the data from each participant’s hypohydrated session was used. Zero-order correlations were calculated between body water measurements and stress-induced changes in cardiac function. Results revealed a significant positive relationship between cardiac output reactivity and intracellular water, extracellular water, and total body water (VanderKaay, Patterson, Shanholtzer, Tulodzieski, et al., 2002). Results also revealed significant negative relationships between total peripheral resistance reactivity and intracellular, extracellular, and total body water. Therefore, these results suggest that individual differences in hydration status may play an important role in cardiovascular function and reactivity. However, the main shortcoming of this analysis is that the relationship between body water and resting cardiac function was not investigated.

Comparatively, Patterson, France, et al. (2002) found similar results between hydration status and both resting cardiac functioning and cardiovascular reactivity.
Thirty-two students participated in a laboratory protocol that included supine baseline, 5-minute posture manipulation (standing), 5-minute viewing of a surgical amputation video, and a 5-minute post video posture manipulation (standing). Bioelectrical impedance measurements of TBW% (percentage of total body water by weight) were obtained at the beginning of each visit. Automated blood pressure and impedance cardiography recordings of heart rate, stroke volume, cardiac output and total peripheral resistance were recorded throughout each period. Zero-order correlations revealed significant inverse relationships between TBW% and resting heart rate and diastolic blood pressure. Reactivity analyses revealed significant negative correlations between TBW% and changes in stroke volume and cardiac output during the first standing task, and between TBW% and stroke volume during the standing task that followed the surgery video. Reactivity analyses further demonstrated significant positive relationships between TBW% and changes in heart rate, diastolic blood pressure, and total peripheral resistance during both standing tasks. Interestingly, no relationships were found between TBW% and surgery video reactivity. The main limitation of this study is that the passive stressor (video) produced essentially no cardiovascular reactivity and was therefore believed to be too weak of a stressor to evoke a response. Hence, research exploring the effect of hydration on hemodynamic functioning and reactivity using a potent stressor is needed.

The aforementioned studies on acute fluid loading and stress demonstrate several important findings and one major limitation. First, different variations of an acute fluid loading protocol affect resting plasma volume. Hence, fluid loading increases the amount
of fluid in the vascular compartment. This fluid was found to increase resting heart rate, cardiac output, and systolic blood pressure, and to decrease total peripheral resistance. Additionally, acute hydration enhancement produced greater cardiac output and total peripheral resistance reactivity, demonstrating a potentially beneficial effect during stress (i.e., less viscous blood with less vascular resistance). Likewise, these studies demonstrated that variations in hydration status are related to differential cardiac function (resting and reactivity). However, these studies did not experimentally manipulate total body water for an extended time period (none > 5 hour), such as several days, which would be more commensurate with ideally hydrated individuals. Given the long-term health consequences of dehydration and the unlikelihood of a person drinking a glass of water immediately preceding psychological stress, this oversight is a major limitation for this area of research. Further, when a person ingests water it primarily moves from the gastrointestinal tract into the vascular compartment; however over time this water will diffuse from the vascular compartment into the extracellular and intracellular space. Therefore these acute fluid loading studies cannot calculate whether increasing a person’s hydration status, by simply having a person drink more water, would result in similar effects on resting and reactivity measurements of plasma volume and cardiac function.

*Long-term Hydration Enhancement*

Although not specifically related to stress, a few researchers have investigated the effect of a long-term hydration enhancement on physiology. Kristal-Boneh et al. (1995) had 9 healthy male volunteers participate in one of two fixed order protocols: control (1-day), heat acclimation (7-day), and heat acclimation plus fluid loading (7-day), or control
(1-day), fluid loading (7-day), and fluid loading plus heat acclimation (7-day). On the control day, participants recorded food and fluid intake. For the hydration enhancement in both protocols, volunteers doubled their daily fluid intake by consuming twice the control day’s amount. Blood samples were taken at the beginning and end of each phase. Results revealed that calculated plasma volume increased during both the heat acclimation and fluid loading phases. Further increases in plasma volume were found when additional fluid intake was combined with heat acclimation. Thus, similar to the acute fluid loading studies, long-term hydration enhancement is related to increased fluid in the vascular compartment which is evidenced by the heightened level of plasma volume.

Likewise, Shore et al. (1988) found a similar effect. In this repeated measures study designed to investigate the effect of hydration on endocrine and renal function, 9 healthy volunteers participated in a 4 phase randomized protocol that included a 5-day baseline, 4-day fluid enhancement period, 3-day intermediate baseline, and a 3-day fluid restriction period. Participants were under a controlled diet for all 4 phases, such that total daily water (food plus fluid) intake was 6.8 liters for enhanced hydration, 1 liter for fluid restriction, and 2.7 liters for both baselines. Participants were instructed to consume their allotted fluid throughout each day. Blood pressure and 24-urine voids were collected daily. Blood samples were collected on the last day of both baselines and daily throughout the fluid manipulation phases. Results indicated that fluid restriction produced greater urine and plasma osmolality when compared to baseline whereas fluid loading decreased urine and plasma osmolality. Significant differences in urine and plasma
osmolality were also observed between the fluid enhancement and restriction periods. These findings demonstrate that long-term hydration enhancement decreases the viscosity of blood, likely resulting from increased hydration in the cardiovascular system. In spite of this discovery, no significant blood pressure differences were found between groups or compared to baseline. Yet, it is possible the small sample size prevented the researchers from detecting such an effect.

In contrast, Patterson and Spinks (2002) did find a relationship between hydration and blood pressure. This study assessed whether participants adhered to a 3-day enhanced hydration regimen determined by bioelectrical impedance. Forty volunteers who reported low daily fluid intake participated in a baseline session, 3-day fluid loading phase, and a follow-up session. During the fluid-loading phase all participants were given six 1-liter bottles of water and instructed to consume 2 bottles gradually throughout each day in addition to their normal fluid intake. Body water and blood pressure measurements were obtained during each session. Results revealed significant negative correlations between systolic blood pressure at follow-up and the change in intracellular water, extracellular water, total body water, and TBW% (percentage of total body water by weight) from baseline to follow-up. Although no significant relationships were found for diastolic blood pressure, the change in TBW% was marginally related to diastolic blood pressure at follow-up. These results suggest that long-term hydration enhancement may be related to lower resting blood pressure. Unfortunately due to the design of this study, it was not possible to assess the effect of hydration on resting blood pressure between fluid enhanced participants and non-fluid enhanced participants due to the lack of a non-
hydrated group. Although this study shows an inverse relationship between hydration enhancement and resting blood pressure assessed in the laboratory, it remains unknown whether enhanced hydration, via increased daily water consumption, affects daily blood pressure in a naturalistic environment.

To summarize, these studies demonstrated that an enhanced hydration protocol increases calculated plasma volume and decreases plasma osmolality. In addition, increases in body water were found to be related to lower systolic blood pressure at rest. Despite these findings between resting hemodynamic function and hydration, none of the aforementioned studies examined the influence of long-term hydration enhancement on cardiovascular reactivity to stress.

In an attempt to explore this phenomenon, Prause et al. (2002) conducted a pilot study using 8 volunteers who were non-randomly assigned to either an enhanced hydration or normal hydration group. Participants in the enhanced hydration group were recruited from the Patterson and Spinks (2002) study after the completion of the fluid loading phase (during follow-up) and were usually tested the following day. The normal hydration group underwent no such protocol. All volunteers participated in a laboratory procedure that included four 5-minute periods: a supine baseline, standing, supine surgery video, and a final standing period. Blood pressure was obtained periodically throughout each period. Change scores (task minus baseline) were computed for systolic blood pressure, diastolic blood pressure, and heart rate to assess group differences in cardiovascular reactivity. Univariate analyses revealed a significant group difference for diastolic blood pressure and mean arterial pressure reactivity during the first standing
period, with the normal hydration group being more reactive than the enhanced hydration group. No significant group differences were found during the second standing period. Results also revealed a significant group difference for heart rate reactivity during the surgery video, with the enhanced hydration group exhibiting greater increases in heart rate. These results are contrary to the Patterson, France, et al. (2002) study which found no cardiovascular reactivity during the passive video stressor. However, that study was investigating correlations between total body water and cardiovascular reactivity whereas this study experimentally manipulated total body water by having participants undergo a 3-day hydration regimen. Therefore, the results of this pilot study suggest that hyperhydration attenuates blood pressure reactivity during postural challenge, but enhances heart rate reactivity during passive stress.

In spite of illustrating that long-term hydration affects diastolic blood pressure during physical challenge and affects heart rate during passive psychological stress, this study has several limitations. Most importantly, this study was not able to utilize a randomized design. This introduces the possibility that individual difference factors may have influenced the results. Second, the sample size was too small to explore the effect of hydration on resting cardiac function or more dynamic measurements of reactivity (e.g. cardiac output, total peripheral resistance). Furthermore, this study also did not measure plasma volume to investigate the effects of chronic hydration on stress-hemoconcentration. Future studies should use a more powerful stressor because this stressor produced virtually no reactivity. Additionally, the participants’ diet was not accounted for during the hydration enhancement phase. Due to the fact that food
contributes to total water intake, it is important to investigate whether heightened water consumption alters the types of food the participant chooses to eat, thus influencing body water. These limitations could easily be addressed in a well designed study. Therefore, the overall goal of the present study was to expand on the results of previous research by designing a protocol that attended to the shortcomings and limitations of these prior studies.

Overview of the Present Study

Although research has shown that acute hydration enhancement affects resting plasma volume and cardiovascular functioning (resting and reactivity to stress), little is known about the effects of long-term hydration enhancement on plasma volume or cardiac function. Prior to the current investigation, it remained relatively unknown whether long-term hydration enhancement influences the cardiovascular system (both at rest and during stress) similar to the cardiovascular effects found during acute hydration enhancement. Thus, the present investigation utilized a 2 X 4 mixed factorial design to assess the relationship between hydration and cardiac function, using Hydration Status (Enhanced Hydration/ Non-Enhanced) as the between subject factor and Task (Math Baseline, Math task, CP Baseline and Cold Pressor Test) as the within subject factor in this design. The dependent variables plasma volume, systolic blood pressure (SBP) and diastolic blood pressure (DBP), heart rate (HR), cardiac output (CO), stroke volume (SV), and total peripheral resistance (TPR), as well as intracellular water (ICW), extracellular water (ECW), total body water (TBW), and TBW% (percentage of total body water by weight) were obtained in healthy volunteers. Additionally, the relationship
between changes in hydration status over a 3-day period and physiologic functioning (24 hour blood pressure and resting plasma volume) were assessed.

The current study had five specific aims to assess the relationship between hydration and hemodynamic function (resting, ambulatory, and reactivity to stress).

**Specific Aim 1:** The present study investigated the effects of a 3-day hydration enhancement on resting HR, blood pressure (SBP and DBP), CO, and TPR in the laboratory. **Hypothesis 1:** It was predicted that individuals assigned to the Enhanced Hydration Group would have significantly lower resting blood pressure and TPR, and greater CO in comparison to the Non-Enhanced Hydration Group.

**Specific Aim 2:** This study examined group (Enhanced Hydration Group vs. Non-Enhanced Group) differences in the magnitude of stress-induced cardiovascular reactivity during acute psychological stress (Cold Pressor Test and mental arithmetic with harassment). **Hypothesis 2:** Enhanced Hydration individuals were expected to have significantly less blood pressure reactivity, greater increases in CO, and a greater reduction in TPR during both the cold pressor and math task.

**Specific Aim 3:** The present study assessed group (Enhanced vs. Non-Enhanced) differences in resting plasma volume and the magnitude of stress-induced hemoconcentration. **Hypothesis 3:** It was predicted that the Enhanced Hydration individuals would have higher plasma volume levels at rest and less stress-hemoconcentration during both the cold pressor and math task. In addition, the Enhanced Hydration individuals were expected to have a greater change in plasma volume over time (Session 2 to Session 3).
Specific Aim 4: This study examined the relationship between changes in body water content (TBW, ICW, ECW, and TBW%) during the hydration enhancement phase (Session 2 to Session 3) and both resting blood pressure (SBP and DBP) and plasma volume (at Session 3). Hypothesis 4: It was anticipated that the change in body water content would be inversely related to blood pressure and positively related to plasma volume after completion of the hydration protocol (Session 3).

Specific Aim 5: The present study assessed the effects of hydration enhancement on 24-hour ambulatory blood pressure (SBP and DBP) and HR over two general time periods: wake period and nocturnal (sleep) period. Hypothesis 5: Individuals in the Enhanced Hydration Group were expected to have significantly lower averaged wake blood pressure and HR in comparison to the Non-Enhanced Group. In addition, it was anticipated that Enhanced Hydration individuals would have lower overall nocturnal blood pressure during the sleep period compared to the Non-Enhanced Group nocturnal blood pressure.

Method

Participants

This study involved 59 university students recruited through undergraduate psychology classes at Ohio University. For inclusion in the study participants were screened to include those who met the following requirements: (a) between 18-30 years of age, (b) in good physical health as indicated by absence of chronic (e.g., cardiovascular disease, cancer, or diabetes) or acute illness (c) no greater than 20% above or below ideal body weight based on the Metropolitan Life Insurance Standards
Height/Weight Tables, (d) report the absence of medications that would affect blood pressure (e.g., beta-blockers, ace inhibitors, antidepressants), (e) report a daily fluid intake less than 1,947 milliliters, and (f) report fruit and vegetable intake no greater than the U. S. Department of Agriculture’s dietary recommendations (less than 10 servings of fruits and vegetables combined; Dixon, Cronin, & Krebs-Smith, 2001). Additionally, only non-smokers were included in this study due to the adverse effects of smoking on hematocrit and plasma volume (Isager & Hagerup, 1971). For each participant, body mass index (BMI) was calculated and participants with a BMI below 19 or above 30 were excluded from the study.

An equal number of male and female participants were recruited for this study. All female participants were scheduled for study sessions during a modified follicular phase of their menstrual cycle (1 to 13 days after the start of menses) to control for hormonal and fluid retention effects on plasma volume and cardiac function (Cullinane, Yurgalevitch, Saritelli, Herbert, & Thompson, 1995; Sita & Miller, 1996). Only women who were not pregnant (as indicated by self-report) were asked to participate. All participants received psychology class credit for each laboratory session (1 credit for each session, except Session 3, which was 2 credits) plus one credit for 24-hour blood pressure monitoring (6 credits total).

Materials

Mass Screening Questionnaire. This survey was used to screen for height, weight, prescription medications, cigarette use, exercise behaviors, and amount of fruits, vegetables, and beverages consumed (see Appendix A). The survey was distributed to
undergraduate psychology students in a packet of questionnaires distributed during mass screening sessions at the beginning of each quarter.

*Health Information Screening Questionnaire.* This form was used to verify health information and to ascertain that only healthy students who reported low fluid consumption were scheduled to participate (see Appendix B).

*Health Profile Survey.* This survey was designed to assess the participants’ self-reported daily fluid intake and diet, health habits (alcohol use, exercise behavior, and sleep habits), barriers to proper hydration (access to safe drinking water, taste, thirst perception), and perceived quality of water at home (see Appendix C).

*24-Hour Food Profile Survey.* This survey was used to verify that participants abstained from food and beverages for 4 hours before the testing session (see Appendix D); any participant who did not comply was not tested. It ascertains whether the participants refrained from engaging in strenuous physical exercise, medication, and drinking alcoholic beverages for 24 hours. In session 3, it recorded self-reported compliance for the enhanced hydration group. Participants who reported non-compliance to the hydration regimen (less than 4 of the 6 liters) were excluded from data analysis.

*Diet Diary.* A 3-day Diet Diary was designed to assess daily food and beverage consumption over a three-day period (see Appendix E). Food and beverage entries for each day were broken down into three time blocks: Awakening through 11:00 AM, 11:00 AM through 5:00 PM, and 5:00 PM through Sleep. During each time block, participants entered the types and portion sizes of all food and beverages consumed during the indicated time-period (e.g., 11:00 AM through 5:00 PM). Participants were asked to
record the items as soon as possible after they eat or drank. Enhanced Hydration individuals were asked to shade in water bottle icon indicating the amount of water consumed during each respective time period.

*Food Serving Chart.* This chart was given to participants to aid in proper completion of the diet diary (see Appendix F). The chart, which was adapted from the Dietary Approaches to Stop Hypertension trial (Sacks et al., 1995), displays examples for each food group with serving sizes.

*Physiological Measures*

*Height and Weight.* Weight was measured on a standard hospital balance beam scale to the nearest quarter pound and height was measured to the nearest centimeter using a stadiometer. The weight measurement was converted to kilograms according to the following formula: \( kg = lbs \times 0.454 \). Using these measurements, body mass index (BMI) was calculated using the formula: \( BMI = \frac{kg}{m^2} \).

*Bioelectrical Impedance.* To assess each participant’s level of hydration, the Multiscan 5000 multi-frequency bioelectrical impedance monitor (Bodystat Ltd, Isle of Man, UK) was used to measure total body water (TBW) and the distribution of extracellular (ECW) and intracellular water (ICW). Bioelectrical impedance measures the resistance and conductance of a mild electrical current, ranging from 5 to 500 kHz, delivered through the participant’s body via electrodes placed on the right hand and foot. The 5 kHz signal has been found to accurately assess ECW whereas the 100 kHz signal is adequate for assessing TBW (Deurenberg, Schouten, Andreoli, & de Lorenzo, 1993: Deurenberg, Tagliahue, & Schouten, 1995). Multifrequency bioelectric impedance
analysis, which ranges from low to high frequencies, has been found to accurately track changes in ICW, ECW, and TBW (Schoeller, 2000). ICW was calculated as TBW minus ECW. Percentage of TBW by weight (TBW%) was calculated from TBW and weight in kilograms.

**Cardiovascular Measures**

*Blood Pressure.* Blood pressure was measured using an automated blood pressure monitoring device (Colin Press-Mate Model BP-8800 sphygmomanometer; San Antonio, Texas). The occluding cuff of the blood pressure monitor was placed over the upper part of the right arm.

*Heart Rate.* Electrocardiograms (ECG) using 3 bipolar silver-silver chloride electrodes was utilized to measure heart rate (HR). A computer program designed to analyze impedance cardiography data (see section below) was used to determine interbeat interval (IBI) for the calculation of HR.

*Stroke Volume.* Stroke volume (SV) was measured using the Minnesota Impedance Cardiograph Model 304B with a tetrapolar band electrode configuration on the neck and thorax (Kubicek, Karnegis, Patterson, Witsoe, & Mattson, 1966). The method is a noninvasive procedure that relates aortic flow to changes in thoracic resistance (Miller & Hovath, 1978). The analysis of impedance cardiography uses a commercially available cardiac output program (COPWIN 5.0; Bioimpedance Technology, Inc).

*Cardiac Output.* Cardiac Output (CO) was measured using the Minnesota Impedance Cardiograph Model 304B. The COPWIN program for impedance
cardiography calculated CO (liter/minute) as the product of mean SV (milliliter) and HR during each minute.

*Total peripheral Resistance.* Blood pressure readings were entered into the COPWIN program after each participant’s data was collected. The program calculated mean arterial pressure (MAP) using the formula: \( \text{MAP} = \frac{(\text{SBP} - \text{DBP})}{3} + \text{DBP} \) and total peripheral resistance (TPR) was then calculated as: \( \text{TPR} \text{ (dyne-seconds/cm}^5\text{)} = \frac{(\text{MAP} / \text{Cardiac Output}) \times 80. \)

*24-Hour Ambulatory Blood Pressure Monitoring.* The 24-hour noninvasive blood pressure recordings was performed with the Spacelabs™ 90217 oscillometric ambulatory blood pressure monitor (Spacelabs Medical, Inc, Redmond, WA). A blood pressure cuff of appropriate size was wrapped around the upper part of the non-dominant arm. The monitor’s internal timer was set to take a reading every 20 minutes between 6:00 AM and 10:00 PM and every 60 minutes between 10:00 PM and 6:00 AM. Participants were instructed to hold the arm relaxed and still at their side each time cuff inflation occurs. Participants were also instructed to indicate on the front page of their 3-day Diet Diary the time that they go to sleep and the time they wake-up during the ambulatory monitoring period.

*Hematological Measures*

**Hematocrit and Biochemical Assays.** All assays were conducted in Ohio University’s Psychophysiology/Health Psychology laboratory. For the determination of the complete blood count (CBC) (includes red and white blood cell count, platelet count,
hematocrit, and hemoglobin), blood was drawn into a 4 milliliter vacutainer tube with EDTA additive. The CBC was determined using a Coulter Counter Model T890.

**Plasma Volume Assessment.** Plasma volume changes was arithmetically calculated from the baseline and manipulation hematocrit (Hct) and hemoglobin (Hgb) values using a method described by Dill and Costill (1974). The formula is as follows:

\[
P_{VA} = (100 \cdot \frac{Hgb_B}{Hgb_A}) - \left[ (100 \cdot \frac{Hgb_B}{Hgb_A}) \cdot \frac{Hct_B}{100} \right],
\]

where Hct_A is the milliliters of red cells per milliliter of blood after mental arithmetic, Hgb_B is the hemoglobin concentration before and Hgb_A is hemoglobin after each task. Hence, the percentage of change in plasma volume (%ΔPV) was calculated as \%

\[%\Delta PV = 100 \left( \frac{P_{VA} - P_{VB}}{P_{VB}} \right),\]

where PV_B (baseline plasma volume) was equal to 100 - the baseline hematocrit.

**Hydration Enhancement**

During the fluid loading phase, participants in the enhanced hydration group was provided with six 1-liter bottles of water. They were instructed to consume two bottles (2 liters) of water per day for 3 consecutive days in addition to their normal daily fluid intake. Participants were asked to drink the water gradually throughout each day. They were told not to include this water intake in their diet entries, but rather indicate consumption by shading the water bottle icon located on each page of the diary.

**Task Description**

**Mental Arithmetic.** Participants performed a 6-minute mental arithmetic task with mild verbal harassment from the experimenter. Participants were instructed to subtract aloud by sevens from a four-digit number as fast and accurately as possible (see
Appendix G for subtraction sheet). The tape-recorded instructions for the task were adapted from Patterson, Krantz, et al. (1995) and were as follows:

The following performance task is similar to the type used in math aptitude examinations. We are interested in physiological changes that occur while performing arithmetic equations in your head. Here are the instructions for the task, please listen carefully. Several times during the next six minutes you will be asked to subtract from a four-digit number by sevens. Your job is to subtract from that four digit number as fast and accurately as you possibly can until I tell you to stop. For example, I may say, Begin subtracting by sevens from the number 1169. You would say, 1169, 1162, 1155, 1148, etcetera, until you are told to stop. During this period we will be recording both the speed and accuracy of your performance. If you do not try just as hard as you can, we will not be able to gather accurate physiological information.

Now here is another important part of the instructions. Several times throughout the task you will be told to stop and begin subtracting by sevens from a new four digit number. Your job again is to subtract by sevens from the new four-digit number as fast as you can. Remember that for our measurements it is important that you keep still so that we can obtain accurate physiological information. If you have any questions ask them now. Remember to work as quickly and as accurately as you possibly can. The task will begin now. Begin subtracting by sevens from the number 1176.
A recorded metronome sound was played continuously throughout the task. Periodically during the task, participants were interrupted and asked to speed up or told to be more accurate each time wrong answers were given.

**Cold Pressor Test.** Participants performed a Cold Pressor Test in which they submerge one of their feet into a bucket of ice water maintained at 4°C (± 1°C) for 3 minutes. The cold pressor was always administered as the last task during Session 3 to minimize physiological carry-over effects.

**Procedure**

An initial screening was conducted at the mass screening session for all introductory psychology students; potential volunteers were contacted by telephone to determine eligibility. All participants were scheduled for three laboratory sessions (see Table 1 for protocol). All laboratory sessions were scheduled at the same time of day in order to accommodate student schedules and each of the main sessions was conducted a minimum of 3 days apart. Session 2 was always scheduled 3 days prior to Session 3 during the same week. To control for any short-term dietary or post-prandial effects on the bioelectrical impedance assessment and hemodynamic measures, participants were asked to refrain from eating or drinking for 4 hours prior to each study session. Participants were also asked to abstain from drinking alcoholic beverages, using recreational drugs, taking any medications, or engaging in strenuous physical exercise for 24 hours prior to each session. One day prior to each testing session participants received a telephone call from the experimenter to confirm appointment time and diet restrictions. Participants also received a call one day prior to beginning the diet diary recordings.
Session 1. After written informed consent (see Appendix H and I) was obtained from each participant, behavioral information such as physical exercise routines and daily fluid intake was recorded (using 24-hour Food Profile Survey). Weight (kg) was measured on a standard hospital balance beam scale and height (cm) was measured using a stadiometer. Participants then laid on an examination table and rest quietly for 20 minutes in order to obtain accurate body water measurements. Electrodes for bioelectrical impedance measures were placed on the participant’s right hand and foot, and a blood pressure cuff was placed on the right arm. Resting blood pressure measurements were obtained during minutes 9, 11, and 13 of the 20-minute rest period and bioelectrical impedance assessments of total body water, intracellular water, and extracellular water was taken during the last 5 minutes. Following the blood pressure and bioelectrical impedance assessment, participants were asked to complete the Health Profile Survey. Next, participants were given a 3-day diet diary and instructed on how to fill it out each day for the three days preceding Session 2. Finally, the Session 2 appointment was confirmed.

Session 2. Upon return to the laboratory, weight, blood pressure, food profile, and bioelectrical impedance measurements were taken as described in Session 1. Next, a 4 milliliter blood sample was obtained for cell blood count (to calculate plasma volume). A nurse performed the blood draw from a vein at the antecubital fossa. Following the physiological assessments, all diet diary entries from the previous 3 days was reviewed with each participant to cover any questions regarding the completion of their diet diary and a new 3-day diet diary was given. Half the participants were randomly selected to
receive six 1-liter bottles of water and instructed to drink 2 bottles of water each day for
the next 3 days along with their normal diet. Finally, the Session 3 appointment was
confirmed.

*Ambulatory Blood Pressure Session.* Male participants were selected for an
ambulatory blood pressure monitoring session. These participants returned to the
laboratory 24 hours prior to their scheduled Session 3. Participants were then fitted with
an ambulatory blood pressure monitor and the blood pressure cuff placed on the non-
dominant arm. Participants were instructed to annotate when they go to sleep and wake-
up and to continue filling out the diet diary. Participants were reminded of their Session 3
appointment. Upon return to the laboratory for session 3, the ambulatory blood pressure
monitor was removed and data downloaded to a computer.

*Session 3.* Upon return to the laboratory, weight, blood pressure, food profile, and
bioelectrical impedance measurements were obtained in the same manner as Session 1
and 2. Following the physiological assessments, all diet diary entries from the previous 3
days were reviewed with each participant to cover any questions regarding the
completion of their diet diary. Next, tetrapolar band electrodes and bipolar silver-silver
chloride electrodes were placed on the participant for resting cardiovascular levels and
reactivity assessments. An intravenous 20-gauge Teflon catheter (Quik-Cath, Travenol
Laboratories, Inc., Deerfield, IL) was inserted into a vein at the antecubital fossa to take
blood samples. A nurse performed all catheter insertions and blood draws. The catheter was
flushed with 5cc infusion saline (0.9% sodium chloride) following each blood draw to keep
the catheter patent. The blood pressure cuff was then placed on the arm opposite the catheter
and connected to the automated blood pressure monitor. The participants then rested quietly in a comfortable chair for a 10-minute Math Baseline period during which cardiovascular measurements (HR, CO, SV, and TPR) were recorded continuously over the last 3 minutes. Blood pressure was taken during minutes 7, 8, and 9 of the Math Baseline. The 6-minute mental arithmetic stressor was presented next. During the math task, blood pressure was measured once every minute and HR, CO, SV, and TPR was recorded continuously throughout the task. Following the math task, participants completed a 10-minute resting recovery period during which HR, CO, SV, and TPR was recorded continuously and blood pressure was taken every other minute (at minutes 1, 3, 5, 7, and 9). Following the recovery period, participants completed a CP Baseline period during which cardiovascular measures (HR, CO, SV, and TPR) were recorded continuously and blood pressure taken once per minute (at minutes 0, 1, 2). After the CP Baseline, participants performed the 3-minute Cold Pressor Test. Blood pressure was recorded at minutes 0, 1, and 2, and cardiovascular measurements (HR, CO, SV, and TPR) were recorded continuously throughout the task period. Blood samples for cell blood count were obtained at four time point during the study session: 9 minutes into the Math Baseline period, 5 minutes into the Math task, 2 minutes into the CP Baseline period, and 2 minutes into the Cold Pressor Test. A total of 16 milliliters of blood was drawn throughout the study session. Following the cold pressor, participants were debriefed (see Appendix J).
Power Analysis

In order to estimate the number of participants needed to have sufficient power to detect viscosity changes during the laboratory stressor, a power analysis was performed using data from previous studies by Patterson and colleagues (Patterson et al., 1993; Patterson, Krantz, et al., 1995) on the means and standard deviations of hematocrit value changes during acute stress to calculate an alpha level of 0.05 and a power level of 0.80 according to standard statistical procedures (Cohen, 1988). Based upon this analysis, it was determined that a minimum of 40 participants were needed to detect viscosity changes due to the laboratory stressor.

However, the abovementioned analysis did not account for the grouping factor (Hydration Group) used in the current study. Therefore, the power analysis was repeated based on data from recent studies of acute stress effects and fluid hydration on hematocrit levels (VanderKaay, Patterson, Shanholtzer, 2002) and cardiac function (Rochette & Patterson, in press). The power analysis utilized the means and standard deviations from these studies. The estimated effect size d values (which indicate difference in standard deviation units) are provided for the main aims of the current study. Specific Aim 1: The smallest d values for group differences on resting cardiac function is for cardiac output (d = .06) and the largest is for systolic blood pressure (d = .35). Specific Aim 2: The smallest d values for group differences on cardiac reactivity to the stress manipulation is for systolic blood pressure (d = .09) and the largest is for diastolic blood pressure (d = .66). Specific Aim 3: The d values for group differences on resting hematological factors are for .27 for hemoglobin and .30 for hematocrit. Thus, the calculated d effects ranged
from low to medium effects in these previous studies. Hence, using an alpha level of .05 and a power level of 0.50, a sample size of 50 participants per group (Enhanced/ Non-Enhanced) would have been needed to detect medium sized effects in this study.

Data Reduction and Analysis

Averages were computed for HR, SBP, DBP, CO, SV, PEP, and TPR for the Math Baseline, Math task, Recovery, CP Baseline, and Cold Pressor Test. Statistical analyses were conducted to assess possible Group (Enhanced Hydration/ Non-Enhanced Hydration) differences in hematological factors and cardiovascular function. This study was initially designed to control for possible gender differences in hydration status. However, because a recent study conducted in our laboratory demonstrated several Hydration Status X Gender effects in cardiovascular measurements (Rochette & Patterson, in press), secondary analyses were conducted to assess whether adding Gender as a predictor to the analyses impacted the original findings or added significant results. An alpha level of .05 was used for all analyses.

Results

Sample Characteristics

Fifty-nine participants entered the study; however, eight participants were lost due to adverse reactions to the blood drawing procedure. Seven participants were lost due to scheduling conflicts, illness or new medication use, and one participant was excluded due to displaying borderline high blood pressure. Consequently, twenty-three males and twenty-two females, between 18 and 23 years of age ($M = 19$) with a BMI ranging from 19.27 to 28.58 ($M = 23.70$), participated in this study. At the initial screening for the
study, participants reported drinking approximately 1.3 liters of fluid on a typical day ($M = 1334.20$ ml, $SD = 349.22$). Male and female participants were equally distributed among the Enhanced ($n = 23$) and Non-Enhanced ($n = 22$) hydration conditions.

**Compliance**

Following the hydration manipulation (Session 3), participants in the Enhanced Hydration Group were asked whether they drank 6 bottles of water. Participants who responded no were asked how many bottles of the water given to them did they actually drink. Results indicated that 20 participants self-reported drinking all 6 liters of water. Only three participants (1 male, 2 female) indicated non-compliance with the hydration manipulation. Both female participants reported drinking 5 liters of water and the male participant reported drinking 5.25 liters of water. A non-compliance rate of less than two-thirds the requested fluid consumption had been set to exclude participants who did not comply with the hydration manipulation, and therefore the three participants were included in all data analyses.

**Resting Cardiovascular Function**

Specific Aim 1 investigated the effects of a 3-day hydration enhancement on resting cardiac function. During each session, an assessment of blood pressure and heart rate were obtained while participants were resting in a supine position (Table 2). The assessments revealed no overall Group differences in SBP, DBP, or HR for participants in the Enhanced and Non-Enhanced Hydration Groups at each of the three sessions. Similarly, no Gender differences were for SBP, DBP, or HR, and no Hydration Group
differences were found when analyzing the data separately for male and female participants.

During Session 3, cardiovascular measures were recorded while participants rested in a seated position (Specific Aim 1) to examine the effects of hydration enhancement on resting cardiovascular measurements (Table 3). The assessments revealed a Hydration Group difference on HR, $t(43) = -2.14, p < .05$, with participants in the Enhanced Hydration Group showing greater HR at rest compared to the Non-Enhanced Group. Hence, there was a Group difference in seated HR but not supine HR. Results also revealed a marginally significant Group difference for CO, $t(43) = -1.77, p = .08$, with participants in the Enhanced Group displaying slightly greater CO at rest relative to the Non-Enhanced Group. Separate analyses for male and female participants investigating cardiac function within each gender revealed no Group differences for hydration status, probably due to reduced sample size within each cell.

**Cardiovascular Reactivity**

*Task baselines.* Due to the presentation of tasks in fixed order (Math always being administered first, Cold Pressor Test second), paired t-test analyses were performed on all resting cardiovascular variables comparing Math Baseline to CP Baseline. Analyses revealed a significant difference between Baselines for SBP, $t(44) = -5.65, p < .05$, DBP, $t(44) = 2.93, p < .05$, HR, $t(44) = -4.17, p < .05$, CO, $t(44) = -4.08, p < .05$, PEP, $t(44) = 2.27, p < .05$, and TPR, $t(44) = 4.88, p < .05$, indicating cardiac function did not return to initial baseline (Math Baseline) values during the CP Baseline. Subsequently, reactivity analyses were performed separately for the Math and Cold Pressor Task using Math
Baseline as the baseline for the Math task and CP Baseline as the baseline for the Cold Pressor Test.

Math reactivity. To ascertain whether there was significant cardiovascular reactivity to the math stressor, paired t-test analyses were performed on all cardiovascular variables comparing cardiac function during Math Baseline with cardiac function during the Math stressor. Results revealed significant differences in cardiovascular function for SBP, $t(44) = -22.48, p < .05$, DBP, $t(44) = -16.39, p < .05$, HR, $t(44) = -12.09, p < .05$, SV, $t(44) = 4.58, p < .05$, CO, $t(44) = -9.09, p < .05$, and PEP, $t(44) = 11.51, p < .05$. There was a marginally significant difference in cardiac function for TPR, $t(44) = -1.66, p = .10$.

Specific Aim 2 of this study examined Hydration Group differences in the magnitude of stress-induced cardiovascular reactivity during acute psychological stress. To assess reactivity to the Math Task, change scores (Math Task minus Math Baseline) were computed on all cardiovascular variables. Univariate ANOVAs for Math Task reactivity revealed significant Hydration Group differences on DBP, $F(1, 43) = 5.18, p < .05$, and MAP, $F(1, 43) = 4.80, p < .05$, with the Non-Enhanced Group displaying greater DBP and MAP reactivity compared to the Enhanced Group (Figure 1). Analyses also revealed a marginally significant Group differences in reactivity for HR, $F(1, 43) = 3.12, p = .09$, and PEP, $F(1, 43) = 3.01, p = .09$, with the Non-Enhanced Group displaying a trend for greater HR reactivity and a greater magnitude decrease in PEP compared to the Enhanced Group (Figure 2 and 3).
To assess the possible influence of gender on these findings, the Univariate ANOVAs for Math reactivity were repeated. Adding Gender to the analyses as a predictor did not change the abovementioned results; however, a significant Hydration Group X Gender interaction emerged for HR, $F(1, 41) = 4.42, p < .05$, revealing no reactivity differences between Enhanced and Non-Enhanced male participants, but a substantial difference in HR reactivity between Enhanced and Non-Enhanced female participants (Figure 4). The difference between Enhanced and Non-Enhanced females, as well as the difference between males and females in the Non-Enhanced Group, was found significant using the Cicchetti (1972) post-hoc procedure ($p < .05$).

**Cold pressor reactivity.** To determine whether there was significant cardiovascular reactivity to the Cold Pressor Test, paired t-test analyses were performed on all cardiovascular variables comparing cardiac function at the CP Baseline to the Cold Pressor Test. Results revealed significant differences in cardiovascular function for SBP, $t(43) = -13.87, p < .05$, DBP, $t(43) = -11.66, p < .05$, HR, $t(43) = -7.50, p < .05$, SV, $t(43) = 3.78, p < .05$, CO, $t(43) = -2.41, p < .05$, and PEP, $t(43) = 4.92, p < .05$, and TPR, $t(43) = -7.21, p < .05$.

To examine Hydration Group differences in the magnitude of stress-induced cardiovascular reactivity during an acute physical and psychological stressor (Specific Aim 2), reactivity change scores (Cold Pressor Test minus CP Baseline) were computed on all cardiovascular variables for the Cold Pressor Test. Univariate ANOVAs for cold pressor reactivity revealed no significant Hydration Group differences on any of the
cardiovascular measurements. Adding Gender to the analyses as a predictor did not change or add any significance finding to these results.

**Hematological Factors**

Failure to obtain complete blood samples on several subjects due to clotting and the inability to locate a vein reduced the sample size for hematological analyses. Consequently, hematological measurements were obtained for 21 males and 19 females at Session 2 and 22 males and 16 females at Session 3.

*Initial Hematological Values.* To ascertain whether there were initial Hydration Group differences in hematological factors, univariate ANOVAs were performed using hematocrit and hemoglobin from the Session 2 blood sample, revealing no significant Group differences at Session 2. Adding Gender to the analyses as a predictor did not alter the aforementioned results but did yield significant Gender main effects for hematocrit, $F(1, 36) = 55.27, p < .05$, and hemoglobin, $F(1, 36) = 30.47, p < .05$ at Session 2, with the males displaying greater values on both measures relative to females (Table 4). No significant Hydration Group X Gender interactions were found.

*Across session hematological change.* Specific Aim 3 assessed Hydration Group differences in the percent change in plasma volume across sessions (Session 2 to Session 3). The univariate ANOVA revealed a significant Hydration Group effect, $F(1, 34) = 4.56, p < .05$, with the Non-Enhanced Group displaying a greater increase in the percent change in plasma volume ($M = 12.72$) compared to the Enhanced Group ($M = 8.04$). Repeating the analysis with Gender as a predictor did not alter or add any significant finding to the results. Yet, one participant displayed a percent change in plasma volume
more than three standard deviations from the mean. Subsequent analysis without this participant revealed only a marginal Hydration Group difference in the percent change in plasma volume ($p = .081$). However, it is unknown whether this change in plasma volume would be unusual with a larger, more representative sample; therefore, the decision was made to keep this participant’s data in all hematological analyses.

*Resting group and gender hematological factors.* Specific Aim 3 also assessed Hydration Group differences in hematological factors, as indicated by hematocrit and hemoglobin values, while participants were resting during Session 3. Univariate ANOVAs revealed no significant Group differences on hematocrit or hemoglobin for participants in the Enhanced and Non-Enhanced Groups during the Math Baseline or CP Baseline.

Adding Gender as a predictor to these analyses did not alter the abovementioned results but did yield significant Gender main effects during the Math Baseline for hematocrit, $F(1, 34) = 96.73, p < .05$, and hemoglobin, $F(1, 34) = 99.65, p < .05$, as well as hematocrit, $F(1, 33) = 88.25, p < .05$, and hemoglobin, $F(1, 33) = 93.06, p < .05$, during the CP baseline. Relative to female participants, male participants displayed significantly greater hematocrit and hemoglobin during the Math and CP Baseline (Table 4). Furthermore, during the CP Baseline there was a significant Hydration Group X Gender interaction for hemoglobin, $F(1, 33) = 4.05, p = .05$, and a marginally significant interaction for hematocrit, $F(1, 33) = 3.76, p = .06$, with Enhanced females displaying greater hemoglobin values over Non-Enhanced females and Non-Enhanced males displaying greater hemoglobin values over Enhanced males. However, Cicchetti (1972)
post test analyses revealed the Hydration Group difference was not significant even though the main effect for Gender still held.

Task Baselines. Due to the presentation of tasks in fixed order (Math always being administered first, Cold Pressor Test second), paired t-test analyses were performed on the hematological measurements comparing Math Baseline to CP Baseline, revealing no significant differences in Baselines. Similarly no Baseline differences were found when conducting the analysis separately by Hydration Group conditions or Gender. Therefore, results indicate hematological function returned to initial baseline values after 12 minutes (at CP Baseline). Despite this finding, reactivity analyses were performed using Math Baseline as the baseline for the Math Task and CP Baseline as the baseline for the Cold Pressor Test to retain similarity to the cardiovascular reactivity analyses.

Reactivity. To ascertain whether there was significant hematological reactivity to the stress manipulations, paired sample t-tests were computed on baseline plasma volume and calculated task plasma volume. Results revealed the Math Task stressor had a significant effect on plasma volume, $t(36) = 2.25, p < .05$; however, there was no significant effect of the Cold Pressor Test on plasma volume.

Specific Aim 3 assessed Hydration Group differences in the magnitude of stress-induced hemoconcentration during both laboratory stressors using pre-task hematocrit and hemoglobin to account for initial hematological values. Univariate ANOVA revealed a marginally significant reactivity difference in the percent change in plasma volume during the Math Task for participants in the Enhanced and Non-Enhanced Hydration Groups, $F(1, 35) = 2.93, p < .10$, with participants in the Non-Enhanced Group
displaying a trend for greater change ($M = -3.65$) compared to the Enhanced Group ($M = -0.42$). However, no significant Hydration Group reactivity differences in plasma volume during the Cold Pressor Task were found. Adding Gender to the abovementioned analyses as a predictor did not yield additional significant findings.

**Bioelectrical Impedance Assessment**

*Body water.* Specific Aim 4 examined body water assessments of TBW, ICW, ECW, and the percentage of TBW by weight (%TBW) among Hydration Group conditions during each session. Independent sample t-test analyses revealed no Group differences in TBW, ICW, ECW, or %TBW at each of the three sessions. Independent t-tests were also conducted to assess possible Gender differences in body water at each session. Results revealed a significant Gender difference in TBW, $t(43) = 12.25, p < .05$, ICW, $t(43) = 14.47, p < .05$, ECW, $t(43) = 8.94, p < .05$, and %TBW, $t(43) = 7.61, p < .05$, at session 1 (Table 5). Similarly, there were significant Gender differences in TBW, $t(43) = 12.27, p < .05$, ICW, $t(43) = 15.45, p < .05$, ECW, $t(43) = 8.87, p < .05$, and %TBW, $t(43) = 5.36, p < .05$, at Session 2 and TBW, $t(43) = 13.19, p < .05$, ICW, $t(43) = 15.95, p < .05$, ECW, $t(43) = 9.41, p < .05$, and %TBW, $t(43) = 9.09, p < .05$, at Session 3. These results indicated males displayed greater body water at each session relative to females. Finally, separate analyses for male and female participants still indicated no Hydration Group differences for body water.

*Relationship between body water and blood pressure.* Specific Aim 4 also examined the relationship between body water content during a laboratory session and resting blood pressure at that respective session. Preliminary analyses revealed
participant weight (kg) covaried strongly with body water measurements (TBW, ICW, ECW, and %TBW), as well as with blood pressure. Subsequently, weight was used as a covariate for all body water analyses in this study. Due to the aforementioned gender differences in body water, first-order partial correlational analyses were conducted separately for male and female participants. Results at Session 1 revealed significant inverse relationships between DBP and TBW, \( r = -0.579, p < .05 \), ICW, \( r = -0.575, p < .05 \), ECW, \( r = -0.537, p < .05 \), and %TBW, \( r = -0.611, p < .05 \), for male participants (Table 6). There were no significant results at Session 1 for female participants. Results at session 2 revealed no significant correlations between body water and blood pressure for male or female participants. Hence, the initial finding between body water and blood pressure for males at Session 1 was not replicated at Session 2.

Due to half of the participants being fluid enhanced during Session 3, separate correlational analyses were conducted by Hydration Group for male and female participants. Results for male participants in the Enhanced Hydration Group revealed a significant inverse relationship between SBP and ECW, \( r = -0.698, p < .05 \), and %TBW, \( r = -0.625, p < .05 \), as well as a marginally significant inverse relationship between SBP and TBW, \( r = -0.570, p = .07 \) (Table 6). No significant relationships were found for Non-Enhanced male participants or female participants in the Enhanced and Non-Enhanced Hydration Groups.

**Across session body water change scores and resting blood pressure.** Specific Aim 4 also assessed the relationship between changes in body water between two laboratory sessions and resting blood pressure at that second session. Again, separate
male and female first-order partial correlational analyses were conducted to assess the relationship between body water change scores from Session 1 to Session 2 and resting supine blood pressure and heart rate at Session 2. Results revealed a significant positive relationship between TBW change (Session 2 minus Session 1) and SBP at Session 2 for male participants, $r = .458, p < .05$, indicating that a greater magnitude decrease in TBW ($M = -.296, SD = .818$) is related to higher SBP at Session 2 (Table 6). A marginally significant inverse relationship was found between TBW change (Session 2 minus Session 1) and HR at Session 2, $r = -.409, p = .059$, indicating a trend between lower HR and a greater magnitude decrease in TBW. No significant relationships were found for female participants.

Additionally, Specific Aim 4 examined the relationship between body water change scores from Session 2 to Session 3 and resting supine blood pressure and heart rate at Session 3. Due to half the participants being fluid enhanced at Session 3, separate male and female first-order partial correlational analyses were conducted separately by Hydration Group. Results for Non-Enhanced male participants revealed a significant positive relationship between HR at Session 3 and the change in TBW, $r = .715, p < .05$, ICW, $r = .725, p < .05$, and ECW $r = .645, p < .05$, between Session 2 and Session 3, indicating that a greater magnitude increase in TBW ($M = .109, SD = .602$) is related to higher HR at Session 3 (Table 6). Results for Enhanced female participants revealed a marginally significant positive relationship between HR at Session 3 and the change in ECW $r = .609, p = .06$, and %TBW $r = .626, p = .053$, between Session 2 and Session 3, indicating a greater magnitude increase in ECW ($M = .109, SD = .315$) is related to
higher HR at Session 3. No significant relationships were found for male participants in the Enhanced Group or female participants in the Non-Enhanced Group.

*Across session body water change scores and resting plasma volume.* Specific Aim 4 examined the relationship between body water change scores (Session 2 to Session 3) and resting plasma volume at Session 3. Calculated Session 3 plasma volume and the across session percent change in plasma volume (Session 2 to Session 3) controlled for initial hematological values using Session 2 hematocrit and hemoglobin. Due to half the participants being fluid enhanced at Session 3, separate male and female first-order correlational analyses were conducted separately by Hydration Group.

Results for Enhanced male participants revealed a significant positive relationship between TBW change (Session 2 to Session 3) and the percent change in plasma volume at Session 3, \( r = .618, p < .05 \), indicating a greater magnitude increase in TBW (\( M = .608, SD = 1.13 \)) is related to a greater magnitude increase in the percent change in plasma volume (\( M = 8.14, SD = 4.78 \)) at Session 3 (Table 6). Results for Non-Enhanced male participants revealed marginally significant positive relationships between Session 3 calculated plasma volume and the change in TBW, \( r = .753, p = .051 \), ICW, \( r = .745, p = .055 \), and ECW, \( r = .733, p = .06 \), from Session 2 to Session 3, indicating a greater magnitude increase in TBW (\( M = .050, SD = .659 \)) and ECW (\( M = .063, SD = .378 \)), as well as a greater magnitude decrease in ICW (\( M = -.013, SD = .295 \)), is related to a greater plasma volume (\( M = 61.77, SD = 2.89 \)) at Session 3. No significant relationships were discovered for female participants in the Enhanced or Non-Enhanced Hydration Groups.
**Ambulatory Blood Pressure**

*Sample Characteristics.* Twenty-six male participants, equally distributed among the Enhanced \((n = 13)\) and Non-Enhanced \((n = 13)\) Hydration conditions, participated in this sub-study of the main investigation. Please note this sample size is larger than the male sample size for the main investigation because three people participated in the sub-study who did not complete the main investigation. Upon entry into the study, the participants’ BMI ranged from 21.10 to 27.97 \((M = 23.96)\) and laboratory assessments revealed an average resting supine SBP of 115.13 \((SD = 7.13)\), DBP of 57.32 \((SD = 4.84)\), and HR of 63.21 \((SD = 8.48)\). Independent t-test analyses revealed no initial Group differences in SBP, DBP, or HR for participants in the Enhanced and Non-Enhanced Hydration Groups.

*Twenty-four hour cardiac function.* Specific Aim 5 assessed the effects of hydration enhancement on 24-hour ambulatory blood pressure (SBP and DBP) and HR over two general time periods: wake period and nocturnal (sleep) period. To assess Group differences in averaged wake and sleep ambulatory cardiac function, averages were computed for SBP, DBP, and HR for the participants’ self-reported wake period and sleep period (Table 7). Independent t-test analyses for sleep revealed a marginally significant Hydration Group difference on HR, \(t(24) = -1.98, p = .059\), with the Enhanced Group displaying greater sleep HR compared to the Non-Enhanced Group (Figure 5). No significant Group differences in averaged wake blood pressure or heart rate were found.

To ascertain whether there were Group differences in the nocturnal decline in SBP, DBP, and HR following sleep, repeated-measure ANOVAs were computed using
the last 3 cardiovascular measurements preceding sleep and the first 5 measurements
during sleep as the within-subject factors and Hydration Group as the between subject
factor. Analyses revealed main effects for Time on SBP, DBP, and HR, $F(7, 98) = 23.98$,
$p < .05$, $F(7, 98) = 15.00$, $p < .05$, and $F(7, 98) = 20.09$, $p < .05$, respectively (Figure 6).
No other significant main effects or interactions were found.

Discussion

The purpose of this study was to examine the effects of long-term fluid
enhancement on hematological factors and cardiovascular function at rest and during
stress. Previous research in the hydration-stress literature has mostly focused on acute
increases in hydration status and does not address the possible influence of long-term
increases in hydration. The goal of the current study was to assess the effects of a 3-day
hydration enhancement on plasma volume and cardiac function (resting, ambulatory, and
reactivity to psychological stress). Additionally, during data collection of the current
investigation, a separate but related study (Rochette & Patterson, in press) found
significant Hydration Status X Gender interactions on several cardiac function
measurements, indicating this current study should also assess the possible role of gender
for each specific aim of the study.

Specific Aim 1

The first objective of the current investigation was to examine the effects of long-
term hydration enhancement on resting blood pressure and HR. Results revealed the
hydration manipulation had no effect on resting systolic or diastolic blood pressure and
that there was no influence of gender on resting blood pressure. Thus, the prediction that
individuals assigned to the Enhanced Hydration Group would have significantly lower supine resting blood pressure in comparison to the Non-Enhanced Group was not supported by these findings (hypothesis 1). Shore et al. (1998) did not find any significant change in blood pressure relative to baseline for either a 4-day water-loading phase or a 3-day water restriction phase. Therefore, the results of the current study together with the Shore et al. study indicate that a change in water intake over several days does not appear to produce any effect on resting blood pressure.

This study also assessed the role of long-term hydration enhancement on resting seated blood pressure and impedance cardiography measures. Participants who received the hydration manipulation exhibited greater resting seated HR. Relative to the supine cardiovascular assessments, the significant seated results are most likely due to gravity causing a greater increase in sympathetic discharge to move the larger volume of blood due to fluid loading. The hydration manipulation did not have a significant influence on any other resting cardiovascular variables. Hence, the results of this study did not support the prediction that individuals assigned to the Enhanced Hydration Group would have significantly lower resting TPR and greater CO in comparison to the Non-Enhanced Group (hypothesis 1). Nevertheless, results did indicate a trend supporting the directionality of the CO prediction. Most likely, this trend is due to the Enhanced Hydration individuals displaying greater HR which would cause an increase in CO assuming SV remains relatively constant.
Specific Aim 2

The second objective of the current investigation was to examine the effects of long-term hydration enhancement on the magnitude of stress-induced cardiovascular reactivity during psychological and physical stress. The results of this study partially support the hypothesis on Hydration Group differences in cardiac function during acute psychological stress (hypothesis 2). Specifically, the prediction that individuals in the Enhanced Hydration Group would display significantly less blood pressure reactivity during math task stress relative to the Non-Enhanced Group was supported by the results. Individuals who underwent the long-term hydration enhancement exhibited attenuated cardiovascular reactivity during the $\beta$-adrenergic (math) stressor. This result is comparable to the findings of a similar investigation which found attenuated DBP reactivity during $\alpha$-adrenergic (cold pressor) stress in individuals who underwent a long-term hydration enhancement (Rochette & Patterson, in press).

On the contrary, the prediction that individuals in the Enhanced Hydration Group would exhibit greater increases in CO and a greater reduction in TPR during Math Task stress was not supported by the results of this study. However, the individuals who underwent the fluid manipulation did exhibit a trend for an attenuated decrease in PEP. PEP is an indicator of myocardial contractility and is commonly used as an index of $\beta$-adrenergic stimulation of the heart (Sherwood, 1993). Due to the inverse relationship between PEP and myocardial contractility, this finding indicates a trend for increased myocardial contractility during stress in the Enhanced Hydration Group (less shortening of PEP = greater contractility) relative to the Non-Enhanced Group. Additionally, there
was a trend for individuals undergoing the long-term hydration enhancement to exhibit attenuated HR reactivity to \( \beta \)-adrenergic stress.

However, the HR trend appears to be driven by Gender X Hydration Group differences in HR reactivity. Male and female individuals who underwent the hydration manipulation tended to exhibit different HR responsivity to the \( \beta \)-adrenergic stress. This is supported by the significant Hydration Group X Gender interaction during the math task. Female participants in the Enhanced Group exhibited substantially less HR reactivity relative to females who did not receive the fluid manipulation. Similarly, Non-Enhanced females had substantially greater HR reactivity relative to male participants.

Stoney, Davis, and Matthews (1987) conducted a meta-analysis on studies that investigated the effects of gender on cardiac function to acute behavioral stressors. They discovered that females show a trend for greater heart rate reactivity to stress relative to males. In other words, the finding that normally hydrated (non-enhanced) females show greater HR reactivity relative to males is a fairly robust finding. Therefore, it is of considerable importance that the current study demonstrates long-term fluid enhancement most greatly impacts the attenuation of HR reactivity among female participants, not male participants.

The current investigation also examined the effects of long-term fluid enhancement on the magnitude of stress-induced cardiovascular reactivity during an \( \alpha \)-adrenergic stressor (Cold Pressor Test). However, results did not reveal any significant findings. Hence, the hypothesis that individuals in the Enhanced Hydration Group would have less blood pressure reactivity, greater increases in CO, and a greater reduction in
TPR during the Cold Pressor Test was not supported (hypothesis 2). One possible explanation as to why the results of this study did not replicate the Rochette and Patterson (in press) finding may be that Rochette and Patterson used a hand immersion cold pressor while the present study used a foot immersion technique. However, Saab et al. (1993) assessed cardiovascular reactivity using three different versions of the Cold Pressor Test: forehead, hand, and foot, and found that all three versions of the Cold Pressor Test resulted in increases in SBP, DBP, and TPR, as well as reductions in SV and CO. With the hand and foot cold pressor HR also tended to increase. With the exception of CO which displayed an increase, the current study demonstrated task main effects of the cold pressor in the expected directions reported by Saab et al., indicating a significant effect of the manipulation. Hence it appears that the hydration manipulation using the foot cold pressor should have resulted in the same Hydration Group cardiovascular effects as the hand cold pressor in the Rochette and Patterson (in press) study.

Nonetheless, one possible explanation as to why the present study failed to show a Hydration Group effect on DBP could be that there are differential effects of gravity on the hands and feet. Pressure in blood vessels under the level of the heart are increased such that the further the distance from the heart, the greater the pressure in the artery or vein. For example, in an upright position the mean arterial pressure in a femoral artery is about 140 mmHg whereas the pressure in an ankle artery is about 180 mmHg (Ganong, 2003). Several compensatory physiological mechanisms, such as an increase in renin, aldosterone, and arteriole constriction help to maintain blood pressure and flow back to the heart. If blood volume is low, these mechanisms become more pronounced (Ganong,
Although this relationship refers to whole body dynamics, these same mechanisms may produce differential effects when stimulated by cold water vasoconstriction in the hand or foot.

**Specific Aim 3**

A third objective of the current investigation was to assess Hydration Group differences in resting plasma volume and the magnitude of stress-induced hemoconcentration. The results of this study do not support the hypothesis that individuals in the Enhanced Hydration Group would have a greater change in plasma volume over time, from Session 2 to Session 3 (hypothesis 3). On the contrary, results support the opposite prediction. The Non-Enhanced Group exhibited the greatest change in plasma volume over the 3-day hydration enhancement phase. However, this significant finding appears to be due to one participant displaying an unusually large increase in plasma volume over the hydration phase. As previously noted, the Hydration Group difference becomes marginal after removing this participant from the dataset, implying that the one outlier is an unusual case that would be less influential if this study had a larger sample size or if the outlier was removed from the data set.

In addition, the prediction that individuals in the Enhanced Hydration Group would have higher plasma volume levels at rest relative to individuals in the Non-Enhanced Group was not supported by the results (hypothesis 3). There were no Group differences in hematocrit, hemoglobin, or plasma volume. Initially it was expected that individuals who underwent the fluid manipulation would exhibit greater plasma volume at rest because studies using an acute fluid loading protocol found such an effect.
(Patterson et al., 2002; VanderKaay, Patterson, Shan Holtzer, 2002). Nevertheless, the lack of support of this hypothesis is logical given the chronic nature of the fluid enhancement unique to this study. With long-term fluid regulation several neurohormonal mechanisms are involved to keep plasma volume at a relatively constant level. When intrathoracic blood volume increases, neurohormonal reflexive actions (e.g., decreasing antiduretic hormone, increasing atrial natriuretic factor, decreasing activity from the renin-angiotensin-aldosterone system) increase kidney water and sodium excretion to return plasma volume towards its normal level (Smith & Kampine, 1990). In the current experiment the initial increase in plasma volume most likely occurred as expected. However the initial increase would also activate long-term neurohormonal controls of plasma volume, which would result in the reduction of plasma volume back to initial values.

Additionally, the prediction that individuals in the Enhanced Hydration Group would have less stress-hemoconcentration during both the math task and cold pressor task was not supported (hypothesis 3). Yet, these results revealed a trend in the predicted direction for the Enhanced Group during the math task, indicating that individuals who did not undergo fluid enhancement had greater plasma volume reactivity. Nevertheless, due to the small sample size and marginally significant findings, no meaningful conclusions can be made from this trend.

Specific Aim 4

Before investigating the fourth objective of this study, a body water assessment was made between genders revealing significant differences on all body water
measurements. This finding is not surprising given that females are commonly found to have lower body water content due to the influence of hormones on fat deposition and synthesis, as well as having lower muscle mass (Berdanier, 2000). Preliminary analyses examined the relationship between body water and blood pressure during the same visit. These analyses revealed an inverse relationship between body water and DBP for males, indicating that lower DBP is associated with higher body water. This finding was not replicated at the participant’s next visit. Yet, this may be due to the decreasing trend in body water between the two visits.

After receiving the fluid enhancement (Session 3), males demonstrated an inverse relationship between body water and blood pressure, indicating lower SBP is related to greater body water. Female participants failed to demonstrate any relationships on these preliminary analyses. This could be attributed to females possessing lower body water content and blood pressure than male participants, as well as hormonal differences which could altogether reduce the strength of any potential relationship between body water and blood pressure. It is also important to note that at Session 3 the sample was divided into four separate categories for analyses (i.e., Enhanced Males, Enhanced Females, Non-Enhanced Males, Non-Enhanced Females). This created small sample sizes ($n \approx 8$) for the correlational analyses, which may make it difficult to find significance for relationships with smaller effect sizes.

The fourth objective of the current investigation examined the relationship between changes in body water content across sessions and resting blood pressure and plasma volume at the session following that time period. Analyses of body water change
across the first two sessions revealed decreases in body water content, where the greater magnitude decrease in body water was related to higher SBP for males. The trend for decreased body water is likely attributable to the second session always occurring on a Monday following the weekend. Most likely, college students are more physically active on the weekends or may consume more diuretics, such as drinking more alcoholic beverages at social gatherings. The relationship of body water change to blood pressure is the same as the relationship found within Session 3 where males who underwent fluid enhancement demonstrated lower body water is related to greater SBP. Additionally, a correlational trend was found between greater reductions in body water across the first 2 sessions and lower supine HR. This indicates that male participants with the greatest decrease in TBW tended to have lower HR.

However, the prediction that the change in body water content would be inversely related to blood pressure after completion of the hydration enhancement protocol was not supported by these results (hypothesis 4). Analyses of body water change between Session 2 and Session 3 revealed no significant relationships between body water change and blood pressure. However, among females who underwent fluid enhancement there was a relationship between greater increases in body water over the fluid loading phase and greater supine HR. Similarly, males who did not partake in the fluid enhancement demonstrated this same relationship between body water and HR from Session 2 to Session 3, which also replicates the trend found in male participants between Session 1 and Session 2.
Given these findings, it is possible to hypothesize the underlying mechanisms leading to greater HR in individuals who possess greater body water or display greater increases in body water change. Increased total blood volume is known to increase the length of cardiac muscle fibers or preload (Ganong, 2003). Greater preload is related to greater contractility of the heart and greater stretch of muscle fibers. The greater stretch of muscle fibers is detected by the atrial stretch receptors of the heart. An increase in the activity of these receptors is known to increase heart rate (Ganong). Usually HR and blood pressure are expected to increase or decrease in similar directions to stimuli or situations. Nevertheless, there are situations where HR and blood pressure change in opposite directions. For example, atrial stretch receptor activation results in tachycardia (elevated HR) and hypotension (Ganong). In the current study, physiological assessments demonstrated this pattern. Yet, it is important to note that HR and blood pressure remained within the normal healthy physiological range for all participants.

Due to blood volume being the hypothesized mechanism between body water and HR it was important to assess whether the change in body water was related to the change in plasma volume. There was partial support for the prediction that the change in body water content would be positively related to plasma volume after completion of the hydration protocol (hypothesis 4). Enhanced male participants exhibited a positive relationship between the change in TBW during the fluid manipulation phase and the across session percent change in plasma volume at the completion of that phase. Male participants who did not undergo the fluid manipulation also exhibited a positive relationship between the change in TBW and ECW and greater plasma volume at the
completion of the manipulation phase. An inverse relationship was also found for ICW and plasma volume. However, the ICW finding is most likely due to a change in the distribution of body water. ICW is the only body water variable that is calculated based on the obtained values of the other two variables (i.e., ECW and TBW). Hence, these results imply that blood volume is increasing with body water.

Specific Aim 5

Finally, the last objective of the present investigation was to assess the effects of Enhanced Hydration on 24-hour ambulatory blood pressure and HR over two general time periods: wake and nocturnal (sleep) period. The results of this sub-study did not support the prediction that individuals in the Enhanced Hydration Group would have lower averaged wake blood pressure and HR in comparison to the Non-Enhanced Group (hypothesis 5). Further, results did not support the prediction that Enhanced Hydration individuals would have lower overall nocturnal blood pressure during the sleep period compared to the Non-Enhanced Group nocturnal blood pressure (hypothesis 5). On the contrary, results were opposite of this prediction indicating that individuals who underwent the fluid manipulation had greater overall nocturnal heart rate. This finding is most likely due to the aforementioned underlying mechanism between blood volume and HR. Lastly, the analysis of nocturnal decline in blood pressure and HR following sleep revealed no overall Hydration Group differences.

Limitations of the Present Study

The current investigation had several limitations that could potentially influence the results of this study and the generalizability of these findings. Most importantly, this
study was conducted over a very homogenous group of people; it only assessed young college students who self reported drinking very few fluids daily. The study used this design to help enhance the effects of the fluid manipulation. However, this is a potential problem because it creates a very narrow distribution of body water in the sample. Thus, when discussing the relationship between cardiac function and body water it is important to remember the range of body water is limited. Consequently, one must use caution in generalizing the results of this study to people who normally consume many fluids. Also, this study recruited college students in order to assess the relationship between cardiac function and body water in young healthy individuals. However, these young adults are likely to have a high metabolic rate compared to older individuals. Hence, it is still unknown whether older adults would have similar cardiovascular responding to the fluid load or in relation to body water.

In addition, the participants in the current study were provided with a fixed amount of spring water regardless of body size. The fixed amount of water was chosen based upon two similar studies (Patterson & Spinks, 2002; Rochette & Patterson, in press), which demonstrated effects due to a long-term fluid manipulation. Yet, people who were smaller therefore had a larger water load per kg and a potentially greater effect of water loading on their physiological systems. Equally important is that the current study provided participants with spring water rather than another beverage containing some electrolyte composition. An isotonic beverage would potentially help with the absorption and retention of fluid during the manipulation phase. However, bioelectrical impedance analysis is unable to accurately detect changes in body water when electrolyte
composition also changes (Thomas, Cornish, Ward, and Jacobs, 1999). The degree of electrolyte composition change and the lag time from fluid ingestion to when a new equilibrium is reached by the body are both unknown. Unfortunately, this study did not assess electrolyte composition of participants which can change due to diet, fluid consumption, and exercise. Due to electrolytes potentially affecting both hydration status and cardiac function, future studies should collect measures of sodium and potassium in addition to the hydration and cardiovascular variables assessed in this study.

The multiple dependent variables and comparisons designed into the current study may pose another potential problem because of the numerous sets of statistical analyses were conducted. The multitude of statistical tests led to an increase in the overall probability of committing a Type I error in this study. Thus, there is an increase in the probability of finding a difference that does not exist. Therefore, one must use caution when interpreting the results of this study and future research is needed to help elucidate the effects of long-term hydration on physiological function during psychological stress.

Another important limitation of the current study was the small sample size used in this investigation. Although sample sizes of 40 participants are often found in psychophysiological research, this study used a multifactoral design which resulted in small cell sizes for the statistical analyses. Recording impedance derived cardiovascular measurements prior to the fluid manipulation would have provided more data between Enhanced and Non-Enhanced Hydration conditions despite the small sample size. However, this study did not assess impedance measurements within individuals across time, which would also be beneficial to rule out individual differences between
participants. Consequently, this is another limitation of the current study that should be attended to in future investigations.

*Directions for Future Research*

An objective of this study was to assess the importance of water in those who do not normally consume a large amount of water. Even so, this is also contrived as a limitation of the study and therefore future studies should be designed to assess cardiac function over a wider distribution of body water. Additionally, because this study was conducted on healthy young adults, it would be interesting to assess body water in different disease populations (e.g., cardiovascular disease, hypertension) relative to a non-diseased population to assess whether differences in body water exist. Stookey (1999) proclaims that a low-water diet is related to some disease states, such as cardiovascular disease. Therefore, assessing the connection of body water to cardiac function in these diseased populations is an important avenue for future research.

Another avenue in this area of research is to expand on the sub-study aspect of the current investigation. Non-dipping status of the cardiovascular system (i.e., no reduction in blood pressure) during sleep is related to increased risk for cardiovascular disease. Kurabayashi et al. (1991) proposed that drinking water at night can reduce the incidence of morning strokes, indicating a relationship between hydration status and morning ischemic events. Hence, it would be important to investigate dipping status in relation to daily wake and nocturnal blood pressure over a larger distribution of hydration status of individuals while accounting for the intensity their of physical activity.
Concluding Remarks

In conclusion, there was partial support for some but not all hypotheses of this study. The current study demonstrated attenuated DBP reactivity during β-adrenergic stress for individuals who received the hydration enhancement. Female participants additionally demonstrated substantially less HR reactivity if they received the fluid enhancement. However, all participants who received the fluid manipulation tended to exhibit greater resting HR. Although there were several other Hydration Group trends in cardiac function, this study failed to demonstrate any other significant findings between the groups for β-adrenergic stress. Moreover, no hydration differences were found during α-adrenergic stress. Even though the reactivity trend indicating more reactivity in the Non-Enhanced Group was in the predicted direction, the current study failed to support any hypothesized Group differences in plasma volume while at rest or during reactivity to the stress tasks.

The current study found body water differences between genders. Results demonstrated correlations between lower blood pressure and higher body water in male participants. However, these relationships did not hold across all visits. This study also consistently demonstrated that non-enhanced male participants showed a relationship between increases in body water and greater HR. Females demonstrate the same relationship only after receiving the fluid enhancement. Additionally, this study showed a positive relationship between plasma volume and an increase in body water across sessions for male participants. Lastly, the current study demonstrated that male
participants who underwent fluid enhancement demonstrated greater overall nocturnal
HR. However, no relationships were found for any other ambulatory assessments.

In short, this study demonstrated limited findings of the hydration manipulation. It appears body water assessments via bioelectrical impedance are a stronger predictor of cardiac function, most specifically HR. Due to the small distribution of hydration status, future studies should be conducted over individuals possessing a wide range of body water states.
References


Table 1. Research Protocol for Participant Visits

<table>
<thead>
<tr>
<th>Pre-session task</th>
<th>Reminder phone call for Session 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Session 1</strong></td>
<td></td>
</tr>
<tr>
<td>Reminder phone call for Session 1</td>
<td></td>
</tr>
<tr>
<td>Informed consent obtained</td>
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<tr>
<td>Height/ weight/ food profile assessed</td>
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<tr>
<td>Resting supine baseline</td>
<td></td>
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<tr>
<td>Blood pressure assessment</td>
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<tr>
<td>Bioelectrical impedance assessment</td>
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<tr>
<td>Health Profile Survey completed</td>
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<tr>
<td>Diet diary and instructions given</td>
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</tr>
<tr>
<td>Appointment confirmed for Session 2</td>
<td></td>
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<tr>
<td><strong>Intersession task</strong></td>
<td></td>
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<tr>
<td>Reminder phone call for beginning diet diary recordings</td>
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<tr>
<td><strong>Pre-session task</strong></td>
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<tr>
<td>Reminder phone call for Session 2</td>
<td></td>
</tr>
<tr>
<td><strong>Session 2</strong></td>
<td></td>
</tr>
<tr>
<td>Weight/ food profile assessed</td>
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<tr>
<td>Resting supine baseline</td>
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<tr>
<td>Blood pressure assessment</td>
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<tr>
<td>Bioelectrical impedance assessment</td>
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<tr>
<td>Blood draw</td>
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<tr>
<td>Diet diary reviewed/ new diary given</td>
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<td>Bottled water given (hydrated group only)</td>
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<td>Appointment confirmed for Session 2 or ambulatory BP session</td>
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<tr>
<td><strong>Pre-session task (ABP group only)</strong></td>
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<td>ABP session (ABP group only)</td>
<td>Fitting for ambulatory blood pressure monitor/ instructions</td>
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<td>Appointment confirmed for Session 3</td>
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<td></td>
</tr>
<tr>
<td>Weight/ food profile assessed</td>
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<td>Resting supine baseline</td>
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<tr>
<td>Blood pressure assessment</td>
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<tr>
<td>Bioelectrical impedance assessment</td>
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</tr>
<tr>
<td>Diet diary reviewed</td>
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<td>Application of electrodes</td>
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<td>Catheter insertion</td>
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<td>10 minute semi-supine resting baseline</td>
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<td>6 minute math task</td>
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<td>10 minute recovery</td>
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<td>3 minute second baseline</td>
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<tr>
<td>3 minute cold pressor task</td>
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<td>Removal of electrodes</td>
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<td>Debriefing and compensation given</td>
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Table 2. Supine Cardiac Function at Each Session by Hydration Group

<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
<th>DBP</th>
<th>HR</th>
</tr>
</thead>
<tbody>
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<td><strong>Enhanced</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>113.70 (6.83)</td>
<td>59.52 (5.56)</td>
<td>64.75 (6.67)</td>
</tr>
<tr>
<td>Session 2</td>
<td>113.14 (6.24)</td>
<td>59.74 (4.58)</td>
<td>64.61 (8.52)</td>
</tr>
<tr>
<td>Session 3</td>
<td>112.96 (8.11)</td>
<td>59.48 (6.71)</td>
<td>64.83 (9.62)</td>
</tr>
<tr>
<td><strong>Non-Enhanced</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>112.06 (8.69)</td>
<td>58.02 (4.35)</td>
<td>61.59 (9.15)</td>
</tr>
<tr>
<td>Session 2</td>
<td>111.27 (6.77)</td>
<td>57.32 (5.36)</td>
<td>60.55 (8.27)</td>
</tr>
<tr>
<td>Session 3</td>
<td>112.41 (7.65)</td>
<td>57.68 (5.11)</td>
<td>60.23 (7.00)</td>
</tr>
</tbody>
</table>

Note: Values are represented as Mean (SD). SBP = systolic blood pressure, DBP = diastolic blood pressure, & HR = heart rate.
Table 3. Session 3 Seated Cardiac Function by Hydration Group

<table>
<thead>
<tr>
<th></th>
<th>Math Baseline</th>
<th>Math Task</th>
<th>CP Baseline</th>
<th>Cold Pressor</th>
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<tr>
<td><strong>Enhanced</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>68.61 (9.24)</td>
<td>91.11 (12.4)</td>
<td>71.25 (8.67)</td>
<td>80.09 (9.24)</td>
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<tr>
<td>SBP</td>
<td>114.84 (9.83)</td>
<td>140.27 (12.1)</td>
<td>118.80 (9.42)</td>
<td>138.79 (10.7)</td>
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<td>DBP</td>
<td>61.49 (7.28)</td>
<td>74.83 (7.10)</td>
<td>59.91 (7.18)</td>
<td>75.83 (9.51)</td>
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<tr>
<td>MAP</td>
<td>79.30 (7.07)</td>
<td>96.62 (7.96)</td>
<td>79.54 (7.24)</td>
<td>96.83 (9.50)</td>
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<tr>
<td>CO</td>
<td>8.35 (1.40)</td>
<td>10.30 (1.84)</td>
<td>8.71 (1.37)</td>
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<td>SV</td>
<td>123.25 (23.9)</td>
<td>113.99 (19.9)</td>
<td>123.56 (23.4)</td>
<td>112.41 (19.2)</td>
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<tr>
<td>PEP</td>
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<td>112.54 (20.9)</td>
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<td>125.79 (14.8)</td>
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<td>TPR</td>
<td>767.37 (198.7)</td>
<td>788.67 (185.8)</td>
<td>733.84 (175.6)</td>
<td>890.13 (196.0)</td>
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<tr>
<td><strong>Non-Enhanced</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>63.24 (7.48)</td>
<td>93.21 (19.9)</td>
<td>68.00 (11.2)</td>
<td>77.75 (14.4)</td>
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<td>SBP</td>
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<td>MAP</td>
<td>78.23 (5.81)</td>
<td>99.33 (8.34)</td>
<td>77.23 (7.35)</td>
<td>92.58 (10.9)</td>
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<tr>
<td>CO</td>
<td>7.69 (1.04)</td>
<td>10.25 (1.97)</td>
<td>8.16 (1.17)</td>
<td>8.73 (1.44)</td>
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<tr>
<td>SV</td>
<td>123.73 (24.8)</td>
<td>113.21 (25.5)</td>
<td>122.13 (22.3)</td>
<td>115.41 (24.7)</td>
</tr>
<tr>
<td>PEP</td>
<td>140.76 (14.3)</td>
<td>109.45 (20.6)</td>
<td>137.03 (17.8)</td>
<td>131.02 (14.5)</td>
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<tr>
<td>TPR</td>
<td>780.33 (147.7)</td>
<td>825.44 (180.1)</td>
<td>730.66 (137.8)</td>
<td>832.59 (172.0)</td>
</tr>
</tbody>
</table>

Note: Values are represented as Mean (SD). HR=heart rate, SBP=systolic blood pressure, DBP=diastolic blood pressure, MAP=mean arterial pressure, CO=cardiac output, SV=stroke volume, PEP=pre ejection period, & TPR=total peripheral resistance.
Table 4. Hematological Factors by Hydration Group and Gender

<table>
<thead>
<tr>
<th></th>
<th>Enhanced</th>
<th>Non-Enhanced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td><strong>Hematocrit</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 2</td>
<td>43.33 (.22)</td>
<td>38.44 (4.27)</td>
</tr>
<tr>
<td>Math Baseline</td>
<td>41.46 (2.48)</td>
<td>35.30 (2.48)</td>
</tr>
<tr>
<td>Math Task</td>
<td>41.40 (2.56)</td>
<td>35.73 (2.48)</td>
</tr>
<tr>
<td>CP Baseline</td>
<td>41.10 (2.92)</td>
<td>35.39 (2.41)</td>
</tr>
<tr>
<td>Cold Pressor</td>
<td>41.88 (2.16)</td>
<td>35.59 (2.60)</td>
</tr>
<tr>
<td><strong>Hemoglobin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 2</td>
<td>15.16 (.75)</td>
<td>13.58 (2.28)</td>
</tr>
<tr>
<td>Math Baseline</td>
<td>14.50 (.89)</td>
<td>12.23 (1.08)</td>
</tr>
<tr>
<td>Math Task</td>
<td>14.48 (.91)</td>
<td>12.42 (1.12)</td>
</tr>
<tr>
<td>CP Baseline</td>
<td>14.38 (1.00)</td>
<td>12.23 (1.06)</td>
</tr>
<tr>
<td>Cold Pressor</td>
<td>14.60 (.91)</td>
<td>12.23 (1.15)</td>
</tr>
</tbody>
</table>

Note: Values are represented as Mean (SD).
Table 5. Body Water at Each Session as a Function of Gender

<table>
<thead>
<tr>
<th></th>
<th>TBW</th>
<th>ECW</th>
<th>ICW</th>
<th>%TBW</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Session 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>40.09 (3.2)</td>
<td>18.61 (1.5)</td>
<td>21.82 (1.8)</td>
<td>54.02 (2.6)</td>
</tr>
<tr>
<td>Females</td>
<td>29.61 (2.5)</td>
<td>14.86 (1.0)</td>
<td>14.75 (1.5)</td>
<td>47.49 (3.1)</td>
</tr>
<tr>
<td><strong>Session 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>39.80 (3.3)</td>
<td>18.19 (1.5)</td>
<td>21.74 (1.8)</td>
<td>55.17 (6.3)</td>
</tr>
<tr>
<td>Females</td>
<td>29.44 (2.2)</td>
<td>14.86 (1.0)</td>
<td>14.58 (1.3)</td>
<td>47.26 (2.8)</td>
</tr>
<tr>
<td><strong>Session 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enhanced:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>39.59 (1.9)</td>
<td>18.07 (.75)</td>
<td>21.53 (1.2)</td>
<td>53.46 (3.0)</td>
</tr>
<tr>
<td>Females</td>
<td>29.86 (2.2)</td>
<td>15.05 (.93)</td>
<td>14.81 (1.3)</td>
<td>46.57 (3.0)</td>
</tr>
<tr>
<td>Non-Enhanced:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>40.79 (3.9)</td>
<td>18.55 (1.8)</td>
<td>22.24 (2.1)</td>
<td>54.90 (2.1)</td>
</tr>
<tr>
<td>Females</td>
<td>29.06 (2.6)</td>
<td>14.69 (1.2)</td>
<td>14.37 (1.5)</td>
<td>47.95 (1.6)</td>
</tr>
</tbody>
</table>

Note: Values are represented as Mean (SD). TBW = total body water, ECW = extracellular water, ICW = intracellular water, & %TBW = percentage of TBW by weight (kg).
Table 6. Significant Body Water Correlations for Males

<table>
<thead>
<tr>
<th></th>
<th>TBW</th>
<th>ECW</th>
<th>ICW</th>
<th>%TBW</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assessments Within the Same Visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>-.579**</td>
<td>-.537**</td>
<td>-.575**</td>
<td>-.611**</td>
</tr>
<tr>
<td>Session 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enhanced SBP</td>
<td>-.570*</td>
<td>-.698**</td>
<td>n.s.</td>
<td>-.625**</td>
</tr>
</tbody>
</table>

| **Across Session (1 to 2) Body Water Decrease** |       |      |      |       |
| SBP                      | .458** | n.s. | n.s. | n.s.  |
| HR                       | -.409* | n.s. | n.s. | n.s.  |

| **Across Session (2 to 3) Body Water Increase** |       |      |      |       |
| Enhanced:               |       |      |      |       |
| %ΔPV                    | .618** | n.s. | n.s. | n.s.  |
| Non-Enhanced:           |       |      |      |       |
| HR                      | .715** | .645** | .725** | n.s.  |
| PV                      | .753*  | .733*  | .745*  | n.s.  |

Note: * p < .08, ** p < .05. TBW = total body water, ECW = extracellular water, ICW = intracellular water, %TBW = percentage of TBW by weight (kg), PV = plasma volume, and %ΔPV = percent change in plasma volume (visit 2-3).
Table 7. Ambulatory Cardiac Function by Time Period and Hydration Group

<table>
<thead>
<tr>
<th></th>
<th>Enhanced</th>
<th>Non-Enhanced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wake</td>
<td>Sleep</td>
</tr>
<tr>
<td>HR</td>
<td>76.96 (9.30)</td>
<td>58.68 (6.62)</td>
</tr>
<tr>
<td>SBP</td>
<td>124.78 (6.45)</td>
<td>104.71 (6.10)</td>
</tr>
<tr>
<td>DBP</td>
<td>73.69 (4.86)</td>
<td>55.85 (4.11)</td>
</tr>
</tbody>
</table>

Note: Values are represented as Mean (SD). HR = heart rate, SBP = systolic blood pressure, & DBP = diastolic blood pressure.
Figure 1. Mental arithmetic change score reactivity for blood pressure by hydration group \((p < .05)\).
Figure 2. Mental arithmetic change score reactivity for heart rate by hydration group ($p = .09$).
Figure 3. Mental arithmetic change score reactivity for pre ejection period by hydration group ($p = .09$).
Figure 4. Mental arithmetic change score reactivity for heart rate as a function of hydration group and gender (*$p < .05$ among non-enhanced participants and for females among enhanced and non-enhanced groups).
Figure 5. Sleeping heart rate as a function of hydration group ($p = .059$).
Figure 6. Ambulatory cardiac function before and after sleep onset (Note: SBP = systolic blood pressure, DBP = diastolic blood pressure, & HR = heart rate. Time main effects on SBP, DBP, & HR are significant, $p < .05$).
Appendix A

Mass Screening Questionnaire

Name: ________________________  Local Phone Number: ____________

Best day of the week and time of day for someone to reach you by phone: ____________

Age: ______  Sex: ________

Height: ________  Weight: _________

Do you smoke cigarettes?  YES  NO (please circle one)
   If yes, how many cigarettes do you typically smoke per day?  ________
   How long have you been a cigarette smoker?  __________
   How long since your last cigarette?  _________________
   Have you recently quit smoking or do you smoke occasionally?  YES  NO
   If yes, how long since your last cigarette?  _________________

Are you currently taking any prescription medication?  YES  NO (please circle one)
   If yes, what medication:  ________________________________________
   For what health reason:  ________________________________________

For the following questions, use your best estimate:

On average, how much of the following do you consume each day?

   Caffeinated coffee/tea: ____________ounces
   Decaf coffee/tea: ____________ounces
   Caffeinated soda: ____________ounces
   Non-Caffeinated soda: ____________ounces
   Juice/sports drinks: ____________ounces
   Water: ____________ounces

On average, how much of the following do you consume each day?

   Fruit: ____________cups
   Vegetables: ____________cups

Note: Count whole fruit/veggies as 1 cup.  Example: 1 apple = 1 cup fruit

Do you exercise regularly?  YES  NO (please circle one)
   If yes, approximately how much time per week do you spend on the following activities?  (please specify minutes or hours)
      Walking/jogging: ____________ (do not include walking to classes)
      Swimming: ____________
      Weight Lifting: ____________
      Aerobics: ____________
      Other (please specify)__________________

IF YOU ARE SELECTED TO PARTICIPATE IN THIS STUDY, THE EXPERIMENTER WILL CONTACT YOU BY TELEPHONE TO GIVE YOU FURTHER INFORMATION.
Appendix B

Health Information Screening Questionnaire

DATE:_________________

STUDY DATE:  S1____ _____________@__________ PM
S2_________________@__________ PM
ABP________________@__________ PM
S3_________________@__________ PM

NAME:____________________________________________

PHONE: ______________________

AGE:_______               Height:__________  Weight___________

DO YOU HAVE ANY PERMANENT OR CHRONIC HEALTH PROBLEMS (e.g. High BP, Asthma, Allergies, Heart Condition, Ulcer, Arthritis, Diabetes, Cancer...):    Y / N
    IF YES, SPECIFY:____________________________________________

HAVE YOU EVER BEEN TOLD THAT YOU HAVE RAYNAUD’S SYNDROME OR HAVE YOU EXHIBITED THE SYMPTOMS OF THIS DISEASE (e.g. Recurrent spasms of the capillaries especially in the fingers and toes upon exposure to cold characterized by pallor, cyanosis, and/or redness, accompanied by pain, that in some cases progresses to local gangrene):   Y / N
    IF YES, SPECIFY:_____________________________________________

DO YOU HAVE ANY HEART RHYTHM PROBLEMS:   Y / N

DO YOU HAVE ANY PHYSICAL DISABILITIES THAT EFFECT GENERAL ACTIVITY LEVELS:    Y / N
    IF YES, SPECIFY:______________________________________________

HAS YOUR HEALTH CHANGED IN THE LAST 6 MONTHS:    Y / N
    IF YES, HOW:_________________________________________________

DO YOU TAKE ANY PRESCRIPTION DRUGS:    Y / N
    IF YES, WHICH:________________________________________________

FOR WHAT HEALTH PROBLEMS:______________________________________

ARE YOU TAKING ANY BIRTH CONTROL OR HORMONES:   Y / N
ARE YOU PREGNANT: Y / N

WHEN WAS THE FIRST DAY OF YOUR LAST PERIOD: ____________________
ARE YOUR MENSTRUAL CYCLES REGULAR: Y / N
HOW MANY DAYS TYPICALLY FALL BETWEEN PERIODS: ________________

DO YOU TAKE ANY NON-PRESCRIPTION DRUGS (e.g. ASPIRIN): Y / N
IF YES, WHAT AND HOW OFTEN: _________________________________
FOR WHAT HEALTH PROBLEM: _________________________________

DO YOU SMOKE: Y / N IF YES, HOW MANY CIG./DAY: ____________

DO YOU EXERCISE REGULARLY: Y / N
IF YES, HOW MANY HOURS A WEEK: __________

DO YOU DRINK COFFEE, TEA, OR CAFFEINATED SODA: Y / N
IF YES, HOW MUCH A DAY: ________________ OZ

HOW MUCH WATER DO YOU DRINK PER DAY: __________ OZ

HOW MUCH JUICE/ SPORTS DRINKS/ OTHER FLUIDS DO YOU DRINK PER DAY: ________________ OZ

HOW MANY CUPS OF FRUITS OR VEGETABLES DO YOU EAT PER DAY: ________________ CUPS
Appendix C

Health Profile Survey

Date: ___________ ID Number: ___________

1. Age: _____

2. Gender:
   ___ Male
   ___ Female

3. Race:
   ___ Caucasian
   ___ African American
   ___ Native American Indian
   ___ Hispanic
   ___ Asian American
   ___ Other

4. Marital Status:
   ___ Single
   ___ Married
   ___ Widowed
   ___ Separated
   ___ Divorced

5. Do you exercise 30 minutes of more:
   (do not include time walking to/ from classes)
   ___ every day
   ___ every other day
   ___ 1 or 2 days a week
   ___ less than once a week
   ___ never

6. Type of exercise you usually engage in:
   ___ walking (other than walking to/ from class)
   ___ jogging (other than jogging to/ from class)
   ___ swimming
   ___ aerobics
   ___ dancing
   ___ weight training
   ___ other
7. On the average, how many servings of the following food groups do you eat each day:

a. Milk, yogurt, and cheese ___ (1 serving = 1 cup of milk or yogurt; or 1½ ounces of cheese)

b. Meat, poultry, fish, dry beans, eggs, and nuts ___ (1 serving = 2-3 ounces of cooked meat, fish, or poultry; or ½ cup cooked dry beans, 1 egg, or 2 tablespoons of peanut butter)

c. Vegetables ___ (1 serving = 1 cup of raw leafy vegetables; or ½ cup of other vegetables)

d. Fruit ___ (1 serving = 1 medium apple, orange, or banana; or ½ cup chopped or canned fruit)

e. Bread, cereal, rice, and pasta ___ (1 serving = 1 slice of bread; or 1 ounce ready-to-eat cereal; or ½ cup cooked cereal, rice, or pasta)

8. How many cups (8 ounces) of coffee or tea do you drink each day? _____

9. How many cups (8 ounces) of fruit or vegetable juice do you drink each day? _____

10. How many cups (8 ounces) of milk do you drink each day? _____

11. How many glasses (8 ounces) of wine do you drink each day? _____

12. How many glasses (12 ounces) of beer do you drink each day? _____

13. How many glasses (8 ounces) of liquor do you drink each day? _____

14. How many glasses (12 ounces) of soft drinks do you drink each day? _____
   What type? __________________

15. How many cups (8 ounces) of water do you drink each day? _____

16. Do you normally drink water when you are thirsty?
   ___ yes
   ___ no
17. If you **do** normally drink water, do you usually drink:
   ___ tap water
   ___ filtered tap water
   ___ drinking fountain water
   ___ bottled distilled water
   ___ bottled spring water
   ___ bottled mineral water

18. What is the main source of your tap water:
   ___ municipal water supply (well water)
   ___ municipal water supply (surface water)
   ___ private property water supply (well water)
   ___ private property water supply (surface water)

19. How would you rank the quality of your tap water?
   ___ Excellent
   ___ Good
   ___ Average
   ___ Marginal
   ___ Poor

20. Do you normally take a shower or bath when you bathe?
   ___ Shower
   ___ Bath

For questions 21-23, please indicate how much each of the statements reflects your feelings about why you do not drink water by circling 0, 1, 2, 3, 4, or 5. The level of feeling is as follows:

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does not bother me</td>
<td>Slightly bothers me</td>
<td>Moderately bothers me</td>
<td>Very much bothers me</td>
<td>Extremely bothers me</td>
<td>Does not apply to me</td>
</tr>
</tbody>
</table>

21. The following is a list of common reasons why many individuals do not normally drink water. **If you do not normally drink water**, please indicate how much each of these reasons reflects your feelings about why you do not drink water when you are thirsty:

   a. Does not satisfy thirst
      
   b. Frequent urination from drinking water
      
   c. Fear of having an accident due to incontinence
      

d. Physical limitation to accessing water
0 1 2 3 4 5
e. Drinking water is not important
0 1 2 3 4 5
f. Just do not think about drinking water
0 1 2 3 4 5

22. The following is a list of common reasons why many individuals do not normally drink tap water. If you do not normally drink tap water, please indicate how much each of these reasons reflects your feelings about why you do not drink tap water:

a. Do not like the smell or odor of my tap water
0 1 2 3 4 5
b. Do not like the taste of my tap water
0 1 2 3 4 5
c. My tap water is turbid or has sediment in it
0 1 2 3 4 5
d. Too many boil order advisories
0 1 2 3 4 5
e. Do not trust tap water purity
0 1 2 3 4 5
f. Do not like the temperature of my tap water
0 1 2 3 4 5

23. The following is a list of common reasons why many individuals do not normally drink bottled water. If you do not normally drink bottled water, please indicate how much each of these reasons reflects your feelings about why you do not drink bottled water:

a. Cannot afford to buy bottled water regularly
0 1 2 3 4 5
b. Do not like the taste of bottled water
0 1 2 3 4 5
c. Do not trust bottled water purity
0 1 2 3 4 5
d. Do not like carrying around water bottles
0 1 2 3 4 5
Appendix D

24-Hour Profile

Subject:__________
Fluid:__________

SESSION 1   Date:__________

1. What and when have you eaten or drank in the last 12 hours?

_____________________________________________________________________
_____________________________________________________________________

2. Have you eaten or drank within the last 4 hours?  Yes___  No___
3. Have you drank any caffeinated beverages (i.e., coffee, tea, soda) or eaten anything containing caffeine (i.e., chocolate) in the past 4 hours?
   Yes___  No___
4. Have you drank any alcohol in the past 24 hours?
   Yes___  No___  If yes, how much?  _____________
5. Have you taken any prescription or non-prescription drugs in the past 24 hours?
   Yes___  No___  If yes, what?  ________________
6. If you exercise regularly, when and for how long did you exercise last?

_______________________________

7. When did your last period start? _______________

SESSION 2   Date:__________

1. What and when have you eaten or drank in the last 12 hours?

_____________________________________________________________________
_____________________________________________________________________

2. Have you eaten or drank within the last 4 hours?  Yes___  No___
3. Have you drank any caffeinated beverages (i.e., coffee, tea, soda) or eaten anything containing caffeine (i.e., chocolate) in the past 4 hours?
   Yes___  No___
4. Have you drank any alcohol in the past 24 hours?
   Yes___  No___  If yes, how much?  _____________
5. Have you taken any prescription or non-prescription drugs in the past 24 hours?
   Yes___  No___  If yes, what?  ________________
6. If you exercise regularly, when and for how long did you exercise last?

_______________________________

7. When did your last period start? _______________
24-Hour Profile

Subject:__________
Fluid:__________

SESSION 3  Date:__________

1. What and when have you eaten or drank in the last 12 hours?
_____________________________________________________________________
_____________________________________________________________________

2. Have you eaten or drank within the last 4 hours?  Yes___  No___

3. Have you drank any caffeinated beverages (i.e., coffee, tea, soda) or eaten anything containing caffeine (i.e., chocolate) in the past 4 hours?  
   Yes___  No___

4. Have you drank any alcohol in the past 24 hours?  
   Yes___  No___  If yes, how much?  ______________

5. Have you taken any prescription or non-prescription drugs in the past 24 hours?  
   Yes___  No___  If yes, what?  ________________

6. If you exercise regularly, when and for how long did you exercise last?  
   ___________________________________________________________________

7. When did your last period start?  ______________

For HYDRATED participants only:

8. Did you drink all 6 bottles of water?  Yes___  No___  
   If no, how many?  __________

9. How many bottles of water did you drink each day?  ______________

10. When did you drink the water? (e.g. throughout the day, all at night, all in the morning)  
    ___________________________________________________________________

11. Do you think you will continue drinking more water than you did prior to this study?  
    Definitely Not   Probably Not   Maybe/Don’t Know   Probably   Definitely
Appendix E

3-Day Diet Diary

Dates: _________________

Days: _________________

24-Hour Blood Pressure Information:
Time you went to bed: __________
Time you woke up: __________

Please record everything you eat and drink 5 pm until the time you go to bed.

<table>
<thead>
<tr>
<th>Food</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grilled chicken</td>
<td>8 ounces</td>
</tr>
<tr>
<td>Soy sauce</td>
<td>1 tablespoon</td>
</tr>
<tr>
<td>Rice</td>
<td>3/4 cup</td>
</tr>
<tr>
<td>Cooked broccoli</td>
<td>1 cup</td>
</tr>
<tr>
<td>Snickers bar</td>
<td>2 ounces</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Beverages</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee</td>
<td>12 ounces</td>
</tr>
<tr>
<td>Iced tea</td>
<td>16 ounces</td>
</tr>
<tr>
<td>Orange juice</td>
<td>8 ounces</td>
</tr>
</tbody>
</table>
Please record everything you eat and drink from the time you woke up today until 11 am.

<table>
<thead>
<tr>
<th>Food</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<tr>
<td>Beverages</td>
<td>Amount</td>
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</tbody>
</table>

Please record everything you eat and drink today from 11 am until 5 pm.

<table>
<thead>
<tr>
<th>Food</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Beverages</td>
<td>Amount</td>
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<td></td>
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<td></td>
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</tr>
</tbody>
</table>
Please record everything you eat and drink 5 pm until the time you go to bed.

<table>
<thead>
<tr>
<th>Food</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<table>
<thead>
<tr>
<th>Beverages</th>
<th>Amount</th>
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<td></td>
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<td></td>
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</tbody>
</table>

Note: Appendix E illustrates an example cover page, sample page, and recording pages for one day. The actually diet diary was a 10.75 cm x 14 cm booklet and contained more recording pages.
Appendix F

Food Serving Chart

When filling out the diet diary, please refer to the following examples. Serving sizes vary. Check product nutrition labels and be specific with your diet entries. When eating or drinking name brand packaged items or fast food, include the name of item and check product for ounces. Beverages without caffeine usually specify “no caffeine.”

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Serving Sizes</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains &amp; Grain Products</td>
<td>1 slice bread</td>
<td>whole wheat bread, english muffin, pita bread, bagel, cereals, grits, oatmeal, crackers, pretzels, popcorn</td>
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<td></td>
<td>1 cup dry cereal</td>
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<td></td>
<td>1/2 cup cooked rice, pasta, or cereal</td>
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<tr>
<td>Vegetables</td>
<td>1 cup raw vegetables</td>
<td>tomatoes, potatoes, carrots, green peas, squash, broccoli, kale, spinach, artichokes, green beans, lettuce, lima beans, sweet potatoes, green beans</td>
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<td>1/2 cup cooked vegetables</td>
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<td>Fruits</td>
<td>1 medium fruit</td>
<td>apricots, bananas, dates, grapes</td>
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<td></td>
<td>1/4 cup dried fruit</td>
<td>oranges, grapefruit, mangoes, melons, peaches, pears, pineapples, prunes, raisins, strawberries, apples</td>
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<tr>
<td></td>
<td>1/2 cup fresh, frozen, or canned fruit</td>
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<td>Diary foods</td>
<td>1 cup yogurt</td>
<td>regular or frozen yogurt, cheese</td>
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<td>1 1/2 oz cheese</td>
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<td>Meats, Poultry, &amp; Fish</td>
<td>3 oz cooked meat, poultry, or fish</td>
<td>beef, chicken, turkey, ham, bacon, fish, roast beef</td>
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<td>Nuts, Seeds, &amp; Dry Beans</td>
<td>1/3 cup nuts</td>
<td>almonds, filberts, mixed nuts, peanuts, walnuts, sunflower seeds, kidney beans, lentils, peas</td>
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<td>2 Tablespoon (Tbsp) seeds</td>
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<td>1/2 cup cooked dry beans</td>
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<td>Fats &amp; Oils</td>
<td>1 teaspoon (tsp) margarine</td>
<td>soft margarine, mayonnaise, salad dressing, vegetable oil</td>
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<td>1 Tbsp mayonnaise</td>
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<td>2 Tbsp salad dressing</td>
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<td>Sweets</td>
<td>1 Tbsp sugar</td>
<td>maple syrup, sugar, jelly, jam, hard candy, jelly beans</td>
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<td>1 Tbsp jelly or jam</td>
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<td>1/2 oz jelly beans</td>
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<td>Fluids</td>
<td>8 oz milk, water, vegetable or fruit juice, lemonade, non-caffeinated soda</td>
<td>milk, buttermilk, apple juice, orange juice, water, sports drink, lemonade, V-8 juice, carrot juice, Gatorade, Sprite, 7 Up</td>
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<td>Caffeinated</td>
<td>8 oz tea, coffee, soda</td>
<td>green tea, cappuccino, latte, iced tea, Coke, Pepsi, Dr. Pepper, Root Beer</td>
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<td>Alcoholic</td>
<td>12 oz beer</td>
<td>beer, light beer, ice beer, long island ice tea, daiquiri, whisky, tequila</td>
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<td>8 oz mixed drink</td>
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<td>1 oz shot (liquor)</td>
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<td>Other</td>
<td>1/2 cup soup</td>
<td>chicken noodle, tomato, rice</td>
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<td>1/2 cup Jell-O</td>
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### Appendix G

#### SUBTRACTION MATH TASK SHEET

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Appendix H

Human Participants Informed Consent (Male)

Title of Research: Resting hemodynamic function and reactivity to acute stress

Principal Investigator: Lynne M. Rochette, B.S.

Thesis Director: Stephen M. Patterson, Ph.D.

Department: Psychology

Federal and university regulations require us to obtain signed consent for participation in research involving human subjects. After reading the statements below, please indicate your consent by signing this form.

Explanation of Study

Previous research conducted by our laboratory has investigated the physiological effects of commercially available rehydration beverages (Performance and Gatorade) and water. The purpose of the present study is to examine the physiological effects of water consumption as well as physiological effects during laboratory tasks and 24-hour blood pressure.

An initial screening procedure was conducted to identify people between the ages of 18 and 30, who are in good physical and mental health. I have been asked to participate because I am in good health. I certify that I have not been told that I have Raynaud’s Syndrome and that I do not exhibit any of the symptoms associated with the disease (recurrent capillary spasms of the fingers and toes upon exposure to cold that is characterized by pallor, cyanosis, and/or redness, accompanied by pain, that in severe cases progresses to local gangrene). I also certify that my fingers or hands do not tend to get white or red when exposed to the cold and that my fingers or hands do not become blue or purplish and painful when exposed to cold.

Qualifying individuals will be invited to participate in the present laboratory protocol that includes 3 or 4 testing sessions scheduled approximately 1-7 days apart:

Session 1 will last approximately 45 minutes and includes:
A 20-minute resting baseline obtained while lying on an examination table
Physiological assessments during the 20 min baseline
Completion of a Health Profile Survey
Assignment of a diet diary to be completed over the three days proceeding the next session (Session 2)
Session 2 will last approximately 45 minutes and includes:
A 20-minute resting baseline obtained while lying on an examination table
Physiological assessments during the 20 min baseline
A registered nurse will collect a small blood sample
Assignment of a diet diary to be completed over the next three days

Ambulatory Blood Pressure Session will last approximately 20 minutes and includes:
Assignment of a blood pressure cuff and monitor to wear continuously for 24 hours before the next session (Blood pressure will be taken 1-3 times per hour after leaving the lab.)

Session 3 will last approximately 1 1/2 hours (90 minutes) and includes:
- 20-minute resting baseline obtained while lying on an examination table
- Physiological assessments during the 20 min baseline
- registered nurse will insert a catheter into the arm for blood sampling
- 10-minute seated resting baseline
- 6-minute math task
- 16-minute resting recovery period
- 3-minute cold pressor test (placing foot in bucket of ice water)

Blood samples will be obtained by a registered nurse during the last 4 tasks

Reminder calls will be made 3 days prior to Session 2 (reminder to begin recording in the diet diary) and the evening before each laboratory session.

Half of all participants will be asked to drink six 1-liter bottles of water, gradually over three days between testing sessions, while the remaining half of participants will not be asked to drink any extra water.

All participants will asked not to eat or drink for 4 hours prior to each testing session and to abstain from drinking alcoholic beverages, using recreational drugs, taking any medications, or engaging in strenuous physical exercise for 24 hours prior to each session to control for possible effects on physiological measures that will be obtained during the course of this procedure.

Physiological variables that will be recorded during this study will include: heart rate, blood pressure, cardiac output (amount of blood pumped by the heart per minute), stroke volume (amount of blood pumped per beat), total peripheral resistance (resistance the blood vessels place on the flow of blood), and bioelectrical impedance measurements. All impedance measurements will be obtained non-invasively using electrodes and transducers attached to the skin. Plasma volume (amount of fluid in the blood stream) will also be assessed. Approximately 2 tablespoons (20 milliliters) of blood will be drawn during the entire study.
**Risks and Discomforts**
There is minimal risk associated with the blood drawing procedure. There is some discomfort associated with the insertion and removal of the needle for the blood draws, the possibility that some bruising may occur, and in very rare occurrences, minor infection. There may be some discomfort due to the water temperature when the foot is placed in the cold water and some redness of the skin may appear; however, these side effects are only temporary. There may also be some slight discomfort and redness of the skin after removing the electrode tape, however these effects are also temporary. In addition, some participants may find the math task challenging and stressful.

**Benefits**
My participation will help researchers to determine the physiological effects of water on cardiac function and plasma volume. The primary benefit to me is I will learn my personal resting blood pressure. Also, I will gain knowledge about research and methodology in the area of Psychophysiology.

**Confidentiality and Records**
I understand that all information obtained from me will be kept strictly confidential, within limits of the law. This information will be identified according to a code number known only to those directly involved with this research project, and any personally identifiable information will be kept in locked files accessible only to those persons directly involved in the study. I consent to the use of my data for research and teaching purposes, and I understand that my identity will not be revealed in any description or publication that results from this research.

**Compensation**
I will receive 6 experimental credits for my participation. One experimental credit will be received for attending session 1, two credits for attending session 2, and three credits for the ambulatory blood pressure session and session 3. I will receive $10.00 for participating in the study upon completion of the laboratory sessions ($5 for completion of Session 1 & 2 and $5 for completion of the remainder of the study protocol).

**Right To Withdraw**
I understand that I am free to refuse to participate in this study or to end my participation at any time, and that my decision will not adversely affect me or cause the loss of benefits to which I might otherwise be entitled.

**Contact Person**
If you have any questions, do not hesitate to ask the experimenter, Lynne Rochette (740-593-0912). You may also contact the director of this project, Dr. Stephen Patterson (740-597-2717), if you have any questions either before or after participating in the study. If you have any questions regarding your rights as a research participant, please contact Jo Ellen Sherow, Director of Research Compliance, Ohio University, (740)593-0664.
**Voluntary Consent**
I certify that I have read and understand this consent form and agree to participate as a subject in the research described. I agree that known risks to me have been explained to my satisfaction and I understand that no compensation is available from Ohio University and its employees for any injury resulting from my participation in this research. I certify that I am 18 years of age or older. My participation in this research is given voluntarily. I understand that I may discontinue participation at any time without penalty or loss of any benefits to which I may otherwise be entitled. I certify that I have been given a copy of this consent form to take with me.

Signature_________________________________________ Date ________________

Witness______________________________________________

Experimenter signature____________________________________________
Appendix I

**Human Participants Informed Consent (Female)**

*Title of Research:* Resting hemodynamic function and reactivity to acute stress

*Principal Investigator:* Lynne M. Rochette, B.S.

*Thesis Director:* Stephen M. Patterson, Ph.D.

*Department:* Psychology

Federal and university regulations require us to obtain signed consent for participation in research involving human subjects. After reading the statements below, please indicate your consent by signing this form.

**Explanation of Study**

Previous research conducted by our laboratory has investigated the physiological effects of commercially available rehydration beverages (Performance and Gatorade) and water. The purpose of the present study is to examine the physiological effects of water consumption as well as physiological effects during laboratory tasks and 24-hour blood pressure.

An initial screening procedure was conducted to identify people between the ages of 18 and 30, who are in good physical and mental health. I have been asked to participate because I am in good health. I certify that I have not been told that I have Raynaud’s Syndrome and that I do not exhibit any of the symptoms associated with the disease (recurrent capillary spasms of the fingers and toes upon exposure to cold that is characterized by pallor, cyanosis, and/or redness, accompanied by pain, that in severe cases progresses to local gangrene). I also certify that my fingers or hands do not tend to get white or red when exposed to the cold and that my fingers or hands do not become blue or purplish and painful when exposed to cold.

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- Physiological assessments during the 20 min baseline
- Completion of a Health Profile Survey
- Assignment of a diet diary to be completed over the three days proceeding the next session (Session 2)
Session 2 will last approximately 45 minutes and includes:
- A 20-minute resting baseline obtained while lying on an examination table
- Physiological assessments during the 20 min baseline
- A registered nurse will collect a small blood sample
- Assignment of a diet diary to be completed over the next three days

Session 3 will last approximately 1 1/2 hours (90 minutes) and includes:
- 20-minute resting baseline obtained while lying on an examination table
- Physiological assessments during the 20 min baseline
- A registered nurse will insert a catheter into the arm for blood sampling
- 10-minute seated resting baseline
- 6-minute math task
- 16-minute resting recovery period
- 3-minute cold pressor test (placing foot in bucket of ice water)

Small blood samples will be obtained by a registered nurse during the last 4 tasks

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**Risks and Discomforts**
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placed in the cold water and some redness of the skin may appear; however, these side
effects are only temporary. There may also be some slight discomfort and redness of the
skin after removing the electrode tape, however these effects are also temporary. In
addition, some participants may find the math task challenging and stressful.

**Benefits**
My participation will help researchers to determine the physiological effects of water on
cardiac function and plasma volume. The primary benefit to me is I will learn my
personal resting blood pressure. Also, I will gain knowledge about research and
methodology in the area of Psychophysiology.

**Confidentiality and Records**
I understand that all information obtained from me will be kept strictly confidential,
within limits of the law. This information will be identified according to a code number
known only to those directly involved with this research project, and any personally
identifiable information will be kept in locked files accessible only to those persons
directly involved in the study. I consent to the use of my data for research and teaching
purposes, and I understand that my identity will not be revealed in any description or
publication that results from this research.

**Compensation**
I will receive 6 experimental credits for my participation. One experimental credit will be
received for attending session 1, two credits for attending session 2, and three credits for
attending session 3. I will receive $10.00 for participating in the study upon completion
of the laboratory sessions ($5 for completion of Session 1 & 2 and $5 for completion of
the remainder of the study protocol).

**Right To Withdraw**
I understand that I am free to refuse to participate in this study or to end my participation
at any time, and that my decision will not adversely affect me or cause the loss of benefits
to which I might otherwise be entitled.

**Contact Person**
If you have any questions, do not hesitate to ask the experimenter, Lynne Rochette (740-
593-0912). You may also contact the director of this project, Dr. Stephen Patterson (740-
597-2717), if you have any questions either before or after participating in the study. If
you have any questions regarding your rights as a research participant, please contact Jo
Ellen Sherow, Director of Research Compliance, Ohio University, (740)593-0664.

**Voluntary Consent**
I certify that I have read and understand this consent form and agree to participate as a
subject in the research described. I agree that known risks to me have been explained to
my satisfaction and I understand that no compensation is available from Ohio University
and its employees for any injury resulting from my participation in this research. I certify
that I am 18 years of age or older. My participation in this research is given voluntarily. I understand that I may discontinue participation at any time without penalty or loss of any benefits to which I may otherwise be entitled. I certify that I have been given a copy of this consent form to take with me.

Signature_________________________________________ Date ________________

Witness__________________________________________

Experimenter signature____________________________________________
Appendix J

Debriefing Statement

Thank you for participating in this research study! In this study, we are examining the effects of long-term fluid enhancement on cardiovascular function during acute stress. We are also interested in the relationship between reactivity and the amount of plasma that is found in the blood stream during stressful tasks.

You completed a serial subtraction math task. This task is often used as a type of mental stressor in plasma volume and cardiovascular reactivity studies. In no way was the math task designed to assess your math skills or ability; it was meant to be a stressful situation. You also completed a cold pressor task by holding your foot in cold water for several minutes. This task is also frequently used in research to assess cardiovascular responses. We needed to put you through a stressful situation (both of these tasks) to determine the effects of increased water consumption on cardiovascular responses and blood plasma during both mental and physiological stress.

The diet diaries were used to measure the amount of water you consumed preceding your physiological tests. At each session we recorded the amount of water in your body. We did not tell you we were recording your body water because we did not want to accidentally influence the amount of fluid you usually consume. Half of the participants in this study received water to drink over the three days preceding this last session. We are attempting to discover if hydration produces different cardiovascular responses during both the math and cold pressor tasks.

Half the participants (all male) were asked to participate in 24-hour blood pressure monitoring to investigate the effects of hydration on blood pressure during the day and night. The reason for only assessed ambulatory blood pressure in the male participants at this time was because the effects of birth control pills and menstrual cycles on body water retention and loss may make it difficult to clearly understand the effect of long-term hydration on heart function. Future research is being designed to investigate this topic using females.

The data that we collected from you today will be kept confidential and in a locked cabinet.

You may experience some redness around your neck and chest because of the electrode bands we applied to you, but this will subside within an hour. Due to the cold temperature of the water, you may have some redness on your foot, but this will also subside.

Is there anything in this study that you suggest we change because we make you feel uneasy?
Do you have any questions at this time?

If at any time you have questions, please feel free to contact Lynne at (740) 593-0912 or visit the health psychology lab (Porter #314).

Because others may be potential future participants in this study, we would prefer that you not tell anyone the purpose of the study or the significant role we are hypothesizing water consumption plays in responding to stress. Of course, you are welcome to tell others what you actually did in the study (for example: placed your foot in ice water) without fear of affecting our future data collection.

Thank you again for participating in this research project.