THE EFFECTS OF DRINKING WATER ON RESTING ENERGY EXPENDITURE

By

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A Thesis
Submitted to the
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of
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LIST OF ABBREVIATIONS

Resting Energy Expenditure ..... REE
Fat-Free Mass ..... FFM
Fat Mass ..... FM
Basal Metabolic Rate ..... BMR
Respiratory Quotient ..... RQ
Volume of Carbon Dioxide ..... VCO_2
Volume of Oxygen ..... VO_2
Sympathetic Nervous System ..... SNS
Autonomic Nervous System ..... ANS
Parasympathetic Nervous System ..... PNS
Resting Metabolic Rate ..... RMR
Blood Pressure ..... BP
Body Mass Index ..... BMI
Total Daily Energy Expenditure ..... TDEE
Muscle Sympathetic Nerve Activity ..... MSNA
Dual-energy X-ray Absorptiometry ..... DXA
Urine Specific Gravity ..... U_{SG}

ABSTRACT

THE EFFECTS OF DRINKING WATER ON RESTING ENERGY EXPENDITURE

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Background: Research suggests that water consumption leads to increased activity of the sympathetic nervous system as shown by increases in heart rate, blood pressure, and increased blood norepinephrine levels. Increased sympathetic activity results in changes in resting metabolic rate (RMR) including energy expenditure and substrate oxidation. Previous research has indicated that water consumption may be used as a means to increase RMR, however the research is limited and inconclusive. The objective of this study is to determine the effects of water ingestion on resting metabolic rate.

Methods: This was a cross-over study of 16 (M=6, F=10) healthy (BMI: 22.8 ± 1.9 kg/m2) adult (age: 25.9 ± 5.5 years) subjects that ingested 250 mL and 500 mL of water after 30 minutes of baseline measures of resting metabolic rate (RMR), the order of water was randomized. RMR was measured using a metabolic cart (**QuarkRMR, Cosmed, Rome, Italy**) and continued for 90 minutes following water consumption. Body composition was assessed using Dual-energy X-ray Absorptiometry (**Horizon, Hologic,**

Bedford, MA). Hydration status was measured using both a urine color chart (**Human Hydration, LLC, Hampton, VA**) and by urine specific gravity (**Fisher Scientific, 13-946-27**).

Results: Our results indicated that water consumption had a metabolic impact. There was a significant ($p=0.022$) increase in resting energy expenditure (50.4 ±115.9 kcal/ 24hr) over the 90-minute period. There was also a significant ($p=0.005$) decrease in respiratory quotient from 0.785 ±0.051 at baseline to an average respiratory quotient of 0.770 ±0.046 over the 90 minutes following ingestion of water. Our results showed a statistically significant ($p=0.003$) 11.4% increase in fat oxidation over the 90-minute period. Both the change in respiratory quotient and fat oxidation were found to be greater after 500 mL of water compared to 250 mL of water ($p<0.05$). There was no significant correlation between hydration status and change in resting energy expenditure.

Discussion: Our results suggest that ingestion of 500 mL of water could provide a feasible method to increasing fat metabolism during a fasting state and may play a role in weight loss. This study was the first to both evaluate and find a dose-dependent effect of water on resting metabolic rate.

CHAPTER ONE: LITERATURE REVIEW

Prevalence of Obesity Among U.S. Adults

The percent of people over the age of 20 in the United States with obesity has increased from 30.5% in the 1999-2002 National Health and Nutrition Examination Survey (NHANES) reports to 35.5% only a decade later.[1] Not only has obesity increased, but all three stages of obesity have increased, with stage 3 obesity doubling between 1988 and 2012. Obesity is associated with increased risk of morbidity and mortality, including a significant risk of death in those with stage 2 or greater. The increased risk of morbidity is apparent by the paralleled increase in diabetes, hypercholesterolemia, and hypertension during this time period.[1] Obesity has been found to be as strong a predictor of poor physical quality of life and increased chronic medical conditions as smoking, heavy alcohol consumption, and poverty.[2] Some research suggest that water consumption may have implications in obesity prevention and weight maintenance.[3–8]

The Physiological Effects of Water

Making up between 55-75% of body weight throughout the life span, water is necessary for life.[9] It is the primary component of cells, tissues, and organs.[10] Water acts as a solvent in the body for ions and is essential for the hydrolysis of proteins, carbohydrates, and lipids.[10] Water serves as a medium for the transportation of nutrients to cells throughout the body and the transportation of waste out of cells. It is also key in the homeostatic control of temperature through evaporation and the loss of heat.[10] Total

body fluid is under tight regulatory control, and is ultimately controlled through urine output and thirst regulation .[9] Water can be consumed in a number of forms with plain drinking water representing the largest part at about 33% of intake, followed by food and other beverages. [11]

Role of Water in Osmotic Pressure and Blood Pressure

Water is key to regulating the osmotic pressure within cells.[12] Osmotic pressure is the amount of force needed to prevent osmosis, or the movement of water across a permeable membrane from a dilute solution to a concentrated solution. It also allows for homeostatic control of the concentration of proteins and ions inside and outside of a cell. Water will move from lower concentrations to higher concentrations, due to osmotic pressure.[12] Osmolality is a measure of the concentration of a solution, taking into account osmotic pressure.[13] It is defined as the number of moles of a particle, or osmoles, dissolved per kg of solvent (osm/kg). This difference in concentration occurs between interstitial fluid and fluid within tissues, specifically in the venule end of the capillaries, forcing water into the capillaries.[12] At the arteriole end of the capillary, blood is highly concentrated and water and small molecules are pushed out of the capillary in a process known as filtration.[12]

When the ventricle walls of the heart contract it causes an increase in hydrostatic pressure, which results in filtration.[12] The highest pressure achieved is the systolic pressure. The lowest pressure before this contraction occurs again represents the diastolic pressure. When fluid balance and hydration status fluctuates, so does blood volume and thus blood pressure. The higher the blood volume, the more hydrostatic pressure that is

needed for filtration, resulting in higher blood pressure.[12] Research has shown that drinking water can result in a subsequent increase in blood pressure. Drinking an extra 30 mL/kg of water throughout the day has been found to cause a statistically significant ($p<0.05$) 2.4 mmHg increase in mean arterial blood pressure in healthy subjects.[14] This is about 2 liters of extra water for a 70 kg man. Another study on 47 patients with orthostatic hypotension resulting from autonomic failure found that 480 mL of water increased the pressor response after only 5 minutes.[15] This increase continued for 30-35 minutes after water ingestion reaching statistical significance ($p<0.05$), and did not level off for over an hour. This significant increase in blood pressure was also seen in older controls, but did not reach statistical significance in young heathy controls. These studies highlight the important role of water in blood pressure regulation.

Hydrolysis of Macronutrients

Water is used during digestion to break down macronutrients into their absorbable form. All three macronutrients (fat, carbohydrate, and protein) go through a process called condensation. With carbohydrates, two monosaccharides form a disaccharide when one molecule loses a hydrogen ion (H+) and the other loses a hydroxyl ion (OH-) and those combine to form water. The opposite occurs during digestion of carbohydrates, the process is reversed and water is added and the disaccharide splits with one molecule taking the hydrogen ion from the water and the other the hydroxyl ion. This process is known as hydrolysis and occurs during the splitting of triglycerides from glycerol in fat and the breakdown of protein into amino acids.

Water's Role in Energy Production

Water is not only needed in the absorption of macronutrients but also in the conversion of those nutrients into energy. Water is needed during the Krebs cycle in order to convert glucose into ATP and hydrogen atoms, which are later used in the electron transport chain. Fat is stored in cells in the form of triglycerides and must be split through hydrolysis in order to leave the cell and be transported to other tissue to be oxidized. Water is crucial to many homeostatic functions throughout the body and research suggests it may also have an impact on the sympathetic nervous system.[15–17]

Water and the Sympathetic Nervous System

Physiology

The Sympathetic Nervous System (SNS) is part of the autonomic nervous system (ANS). The autonomic nervous system is responsible for homeostasis during environmental stress.[18] This system regulates the body continuously without conscious effort.[12] It is made up of two divisions: the parasympathetic nervous system (PNS) and sympathetic nervous system (SNS), which in many cases act as an antagonist of one another **(Figure 1**).[18] The SNS is stimulated by the hypothalamus, which results in a fight or flight response, while the PNS is active during times of rest, and acts counter to the SNS in order to return to rest after stress.[12] Each division of the ANS has two types of neurons, pre-ganglionic and post-ganglionic. Pre-ganglionic neurons either have axons that connect directly to the medulla of the adrenal glands, or they connect with post-ganglionic neurons. Post-ganglionic fibers terminate on the smooth muscle of blood vessels, hair follicles, sweat glands, and visceral organs.

There are two main neurotransmitters released by the sympathetic nervous system and these nerve fibers. The first is released by cholinergic neurons and is called acetylcholine. Acetylcholine is present in all pre-ganglionic neurons, and in the post-ganglionic neurons that affect the sweat glands and produce vasodilation within skeletal muscle. The second neurotransmitter, norepinephrine, is released by noradrenergic neurons which make up all of the post-ganglionic neurons, except for those just mentioned.[18] The sympathetic nervous system works on the medulla of the adrenal glands by stimulating the release of epinephrine and norepinephrine.[12] The release of norepinephrine from post-ganglionic cells acts on differing types of adrenoceptors on target organs.[18] The effect of the neurotransmitters on these receptors is dependent on which type of adrenoceptors is present on the target organ and the location of the receptor.[19] This action causes physiological changes such as increased heart rate, dilated pupils, increased blood pressure, constricted blood vessels, and elevated plasma glucose and fatty acid levels.[18]

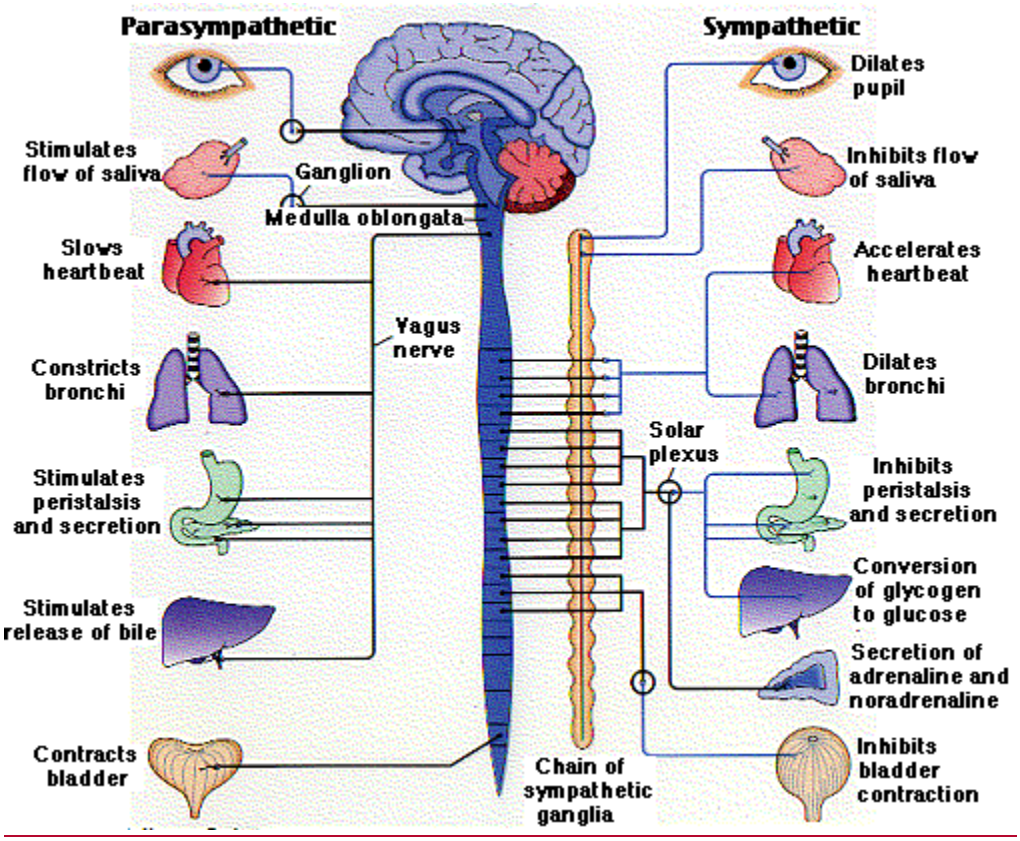

Figure 1 Comparison of the Effects of the Sympathetic and Parasympathetic Nervous System on Target Organs[20]

Mechanisms Behind Water's Effects on the Sympathetic Nervous System

Pressor Response

As previously discussed 480 mL of water was found to significantly ($p<0.0001$) increase BP in patients with orthostatic hypotension caused by autonomic failure, as well in older adults controls ($P<0.001$).[15] This study also found a significant ($P<0.05$) increase in plasma norepinephrine in older controls, and a non-significant increase in plasma norepinephrine in 6 of 7 younger

controls. The increase in plasma norepinephrine in young controls was not seen during ganglionic blockade. In those with autonomic failure, the pressor response to water was eliminated during ganglionic blockade. The increase in norepinephrine that coincided with the increased pressor response, and the lack of change during ganglionic blockade, represents a crucial role of the SNS in the pressor response following water consumption.[15]

Osmolality

Research has also found that stimulation of the sympathetic nervous system after drinking fluids may be dependent on if the beverage is hypo osmotic (lower osmolality than the fluid within the body), or hyper osmotic (higher osmolality than fluid within the body).[16,17] A study on baroreflex impaired mice showed that ingestion of saline solution did not elicit the same increase in blood pressure that was seen following water, suggesting that the change in blood pressure is caused by a change in osmolality. The literature demonstrates that these changes were regulated by the sympathetic nervous system by blocking adrenoceptors in the mice and preventing the activity of norepinephrine, resulting in no changes in blood pressure.[16]

Similar results were found in a cross-over study involving humans.[17] Each subject ingested both water and saline solution, but only water led to a significant ($p=0.01$) decrease in heart rate and a significant ($p<0.01$) increase in total peripheral resistance and baroreflex sensitivity. There were no significant changes in blood pressure in either group,[17] but this has been found in previous research on young, healthy subjects, despite increased sympathetic activity.[15] These studies suggest that the increase in

sympathetic activity could be related to hypo-osmolality. The activation of the sympathetic nervous system through consumption of water is important in obesity research due to the potential for increased energy expenditure during sympathetic activation.

Energy Expenditure

Drinking water does not only act as substitute for high caloric beverages,[21] but also may directly aid in energy balance through an increase in resting energy expenditure.[6–8,22] An increase in resting energy expenditure (REE) would contribute to total daily energy expenditure (TDEE) and impact energy balance. Energy balance can be positive, in which you take in more energy from food and beverages than expended, or negative, in which you expend more than you take in. Positive energy balance results in weight gain, whereas negative energy balance results in weight loss.[13]

Total Daily Energy Expenditure

Total daily energy expenditure is the total energy requirement of the body during a 24-hour period and it is made up of four components. Basal metabolic rate (BMR) is a measure of the bare minimum amount of energy needed to sustain life and is therefore measured under very controlled conditions, including control of environmental temperature. While BMR and REE differ, both are contributors to TDEE and influence energy balance. Since these conditions are difficult to achieve RMR is measured, which is BMR plus a thermogenic effect of the non-standard conditions.[13]

In sedentary individuals RMR accounts for 65-80% of TDEE, depending on activity level, and is a measure of the energy required when a person has fasted and is in

a comfortable relaxed, state. RMR can then be converted to a 24-hour measure of energy expended. Thermic effect of food (TEF) involves the energy required to digest, absorb, transport, metabolize, and store fats, carbohydrates, and proteins.[13] There is much variation within an individual and between individuals when calculating the TEF depending on the type of nutrient that is being oxidized. Protein has the greatest thermic effect, followed by carbohydrate and last fat, but generally they are together thought to contribute to about 10% of TDEE.[13] Physical activity can contribute up to 20-40% of TDEE, but this is easily varied and can be much lower or higher depending on if a person is extremely sedentary or physically active, including up to 80% in extreme in athletes, such as those participating in the Tour de France.[23] The last component of TDEE is thermoregulation and represents a very small percent of TDEE. Thermoregulation is a homeostatic mechanism meant to maintain the body's core temperature.[13] REE accounts for the majority of energy needs, and an increase in REE would provide a practical way to achieve energy balance and prevent weight gain.

Resting Energy Expenditure

REE can be measured by either direct or indirect calorimetry.[24] Direct calorimetry measures the amount of total metabolic heat produced. This involves a specialized enclosed chamber, which measures heat directly. Indirect calorimetry requires a gas analyzing metabolic cart, and yields results that closely match those of direct calorimetry. The principal behind indirect calorimetry relies on the notion that skeletal muscle cellular respiration (energy metabolism) utilizes oxygen and produces carbon dioxide. REE can be measured via indirect calorimetry through the proportions of oxygen and carbon

dioxide during external respiration. There are two methods for measuring gas exchange: closed circuit and open circuit. Closed circuit indirect calorimetry involves a spirometer containing 100% oxygen gas. Following expiration, carbon dioxide (VCO_2) is removed and oxygen (VO_2) is measured based on the amount of oxygen inspired from the spirometer. In open circuit calorimetry, surrounding air is inhaled and then analyzed as it passes through a two-way valve.[24]

One of the most influential factors influencing REE is body composition.[25] While those that are obese tend to have a higher REE than healthy weight individuals,[25,8] research has shown that fat-free mass (FFM), and more specifically, muscle mass, not fat mass (FM), is the strongest predictor of REE.[25] Similarly, research has shown that the metabolic rate of resting muscle is positively correlated with 24 hour REE.[26] The same study found that forearm oxygen consumption has a statistically significant ($p<0.005$) positive correlation with both forearm muscle volume and basal metabolic rate (BMR).[26] Basal metabolic rate is similar to REE but it is measured after a long fast of at least 12 hours, in a lying down position, and is best measured immediately following waking up.[13]

Respiratory Quotient

Respiratory quotient (RQ) is a variable which is derived from the proportion of expired carbon dioxide in relation to inspired oxygen (VCO_2/VO_2).[13] RQ can indicate which substrate is being metabolized, carbohydrates, fats, or proteins. However, it cannot tell us where the oxidation is occurring (muscle, liver, adipose tissue, etc.). When metabolizing carbohydrates, glucose is converted into two pyruvate molecules.[13] When sufficient oxygen is available each pyruvic acid is converted to acetyl CoA upon entry

into the mitochondria of a cell. Acetyl CoA enter the Krebs cycle, where ATP, NADH, FADH, and CO_2 is produced. The NADH and FADH enter the electron transport chain where oxidative phosphorylation occurs. This requires a large amount of oxygen to be present in order to accept hydrogen ions along the electron transport chain. When fat is metabolized it is broken down into glycerol and 3 fatty acids. Each fatty acid is then converted into many acetyl CoA, dependent on the number of carbons within the fatty acid. The large amount of acetyl CoA produced from a fatty acid results in a larger amount of H^+ ion being stored as NADH and FADH, and further entering the electron transport chain, in comparison to glucose (carbohydrate).[13] Therefore, a greater amount of oxygen is needed when metabolizing fats than carbohydrates. During glucose metabolism six total carbon dioxide molecules are produced (3 per pyruvate molecule). One CO_2 molecule is produced during the conversion of pyruvate to acetyl CoA and two during the Krebs cycle. During fat oxidation the amount of CO_2 produced is again dependent on the number of carbon bonds within the fatty acid and the amount of acetyl CoA produced.[13] It is these differences in oxygen utilization and carbon dioxide production that allows for the estimation of carbohydrate and fat oxidation based on the ratio of expired CO_2 and inspired O_2, or respiratory quotient (**Equation 1**). When oxidizing carbohydrates this ratio is 1:1, or an RQ value of 1.0. The RQ value of fat is dependent on type of fatty acid being oxidized but typically has a ratio of 18:25, or an RQ of 0.72.

Equation 1 Calculating Respiratory Quotient

Carbohydrate Oxidation **Respiratory Quotient**

$C_6H_{12}O_6 + \mathbf{6\ O_2} \rightarrow \mathbf{6\ CO_2} + 6\ H_2O + Heat$ $6\ CO_2 : 6\ O_2 = 1$

Carbohydrate

Fat Oxidation

$C_{18}H_{32}O_2 + \mathbf{25\ O_2} \rightarrow \mathbf{18\ CO_2} + 16\ H_2O + Heat$ $18\ CO_2 : 25\ O_2 = 0.72$

Fat

Urine needs to be collected during indirect calorimetry to account for protein oxidation.[13] Nitrogen, as well as trace amounts of oxygen and carbon dioxide, is excreted as urea and must be accounted for as a loss of energy. The left over amino acid chain can then be oxidized and used for energy. For every 1g of nitrogen in the urine, 6L of oxygen is inhaled and 4.8L of carbon dioxide is exhaled. Protein then has an RQ of about 0.8.[13]

Using these ratios, it can be estimated whether someone is oxidizing more fat (closer to 0.7) or carbohydrates (closer to 1.0).[13] A typical morning, fasting RQ in those on a balanced diet is 0.8.[27] Established tables can be used to predict the % of fat and the % of carbohydrate being metabolized for fuel based on RQ and to predict corresponding caloric values based on 1L of inhaled oxygen or 1L of exhaled carbon dioxide. [13]

Respiratory Quotient, Resting Energy Expenditure, and Obesity

Many studies have found an association between obesity and both resting energy expenditure and respiratory quotient.[27–31] Research on Pima Indians has

found that both low resting metabolic rate ($p=0.016$)[30] and high respiratory quotient ($p<.0.01$) are associated with future weight gain.[27] A study with 33 overweight, premenopausal women found that both low fat oxidation and high carbohydrate oxidation following a meal predicts weight gain in the coming year or two ($p<0.05$).[28] High RQ has also been found to predict weight gain in non-obese women.[31] Similarly, in type 2 diabetic women it has been found that a significant increase in RQ and a significant decrease in REE was predictive of weight gain in the following 3 months.[29] Research supports both an increase in REE and a decrease in RQ as approaches to aid in obesity prevention. A known method to increasing REE is through stimulation of the sympathetic nervous system as described below.

Mechanisms for Increased REE Through Sympathetic Activation

The sympathetic nervous system impacts resting energy expenditure through the release of catecholamines.[32] Catecholamines are a group of chemicals that activate receptors within the autonomic nervous system **(Figure 2)**.[19] Catecholamines regulate glucose synthesis and use, the mobilization of fat, and protein metabolism.[32] Of particular importance in the sympathetic nervous system is norepinephrine.[19] When the fight or flight response is initiated, norepinephrine acts on the liver to produce more glucose, through gluconeogenesis and increased glycogenolysis, to provide a large pool of glucose in the blood, which is necessary for quick anaerobic ATP production. The liver also increases fatty acid oxidation and catecholamines act on the a_2-adrenergic receptors of β-

cells to prevent insulin secretion. Norepinephrine acts on adrenergic receptors to cause vasoconstriction, making it a key regulator of blood pressure.[19]

Catecholamines act on β-adrenergic receptors throughout the body to promote thermogenesis.[33] Thermogenesis is the production of heat[34] and is a result of enzymatic reaction within cells, digestion and absorption of nutrients, and homeostatic mechanism meant to adapt to cold weather or exercise.[8] Sympathetic nerves in white adipose tissue contribute to thermogenesis by stimulating enzyme activity involved with lipid mobilization and is estimated to account for up to 7% of REE.[34] Noradrenaline limits the uptake of triglycerides into white adipose tissue by regulating lipoprotein lipase,[35] which is responsible for the breakdown of triglycerides to fatty acids for transport into adipose tissue. This is supported by research that has found that thermogenesis within the white adipose tissue is lowest among those with obesity.[36] The SNS also promotes non-shivering thermogenesis in brown adipose tissue.[37] Brown adipose tissue is stimulated by the sympathetic nervous system in response to cold stress and ingestion of food.[13] It has a high amount of mitochondria with uncoupling proteins. Uncoupling proteins allow H^+ ions to cross through the electron transport chain resulting in the loss of heat, as opposed to production of ATP.[13] Brown adipose tissue has been found to increase a child's metabolism by as much as 100%, but adults have very little brown adipose tissue and non-shivering thermogenesis only increases metabolism by less than 15% when stimulated.[37] Catecholamines have been found to increase muscle thermogenesis by increasing oxygen consumption within muscle tissue.[32] They also acts on the β-receptors of skeletal muscle to increase the activity of LPL and the supply of free fatty acids for use

by the muscles.[35] Through increased thermogenesis and nutrient mobilization, the SNS is a potential regulator of REE.

Conclusion

Despite the essential role of water to life, national representative data has shown that 24% of Americans over the age of two fail to drink any plain water during a typical day.[11] Water is often times displaced by soft drinks or juices, which is associated with an increased risk of both high body mass index (BMI) and type 2 diabetes due to the high amount of sugars.[3] There is an increasing rate of obesity in the U.S., and an increase in water consumption could potentially slow this trend. Water does not only have less sugar than many other beverages, but it is necessary to many physiological functions of the human body. One of which may be an increase in resting energy expenditure through stimulation of the sympathetic nervous system. The purpose of this study is to examine the effects of water consumption on resting metabolic rate and its implications in obesity research.

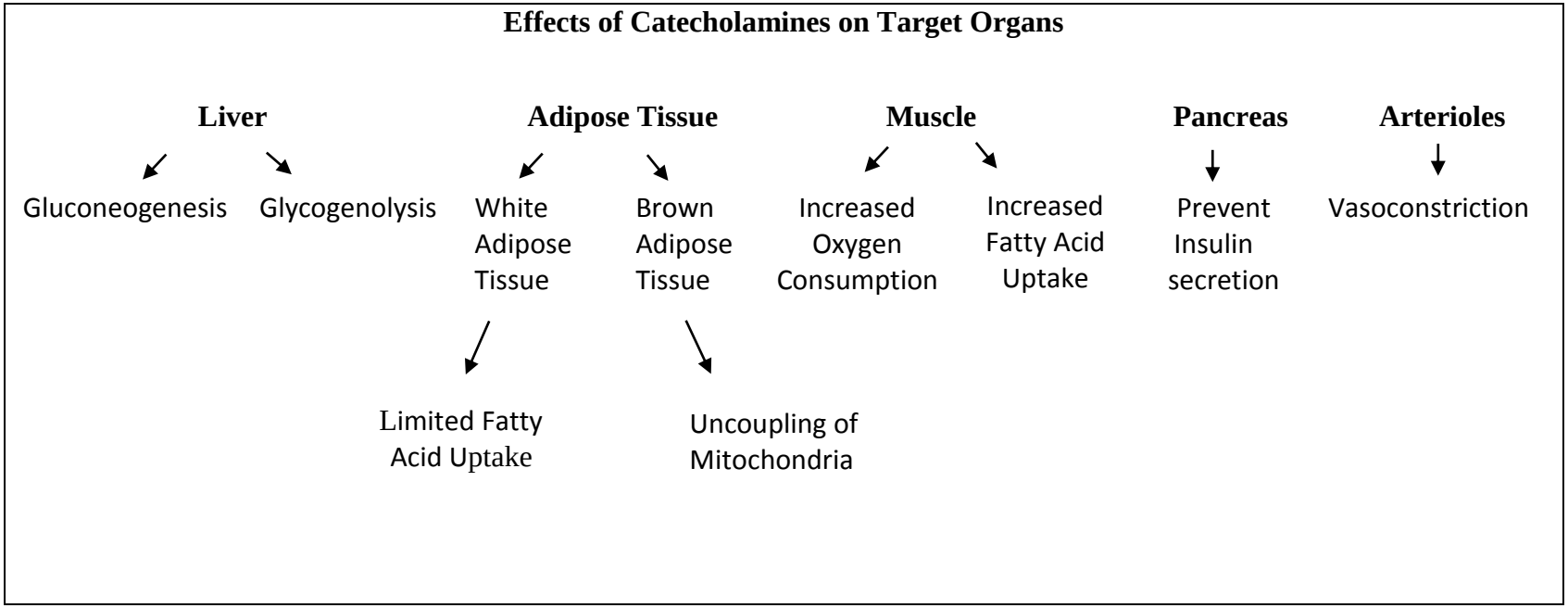

Figure 2 Effects of Catecholamines on Target Organs in Sympathetic Activation

CHAPTER TWO: RATIONALE AND GAPS IN LITERATURE

Rationale

While water has been shown to increase the activity of the sympathetic nervous system,[15–17] and the sympathetic nervous system has been shown to increase REE,[32–34] very few studies have looked at the effects of water consumption on REE. Previous studies have yielded a variety of results with one study showing a 30% increase in REE after ingesting 500 mL of water,[7] and two studies showing a 3.6% increase after 500 mL and 5% increase in REE after ingesting 7.5 mL/kg of water, only after ingesting 3°C water.[38] A study comparing obese women and normal weight women found a 20% increase in those that were obese, and 12% increase in those within a healthy weight after ingesting 1000 mL of water.[8] Last, a study on overweight and obese children found a 25% increase in REE after drinking 10 mL/kg of water.[6] The resulting changes in RQ following ingestion of water are just as varied as REE, with a trend toward a decrease in RQ in those that are healthy[7], and no changes in those that are overweight or obese.[39,8]

There are many factors which may explain the drastically different results between these studies. The effects of water on resting energy expenditure is not only limited by the quantity of literature on that topic but the quality. Four of the seven studies had 16 or less subjects.[39,38,7] The two studies with the least amount of subjects (8 and 12) were the only studies to find no increase in REE after drinking room temperature water.[38,39] It is possible that the small sample size prevented these studies from reaching

statistical significance. Based on a similar study which found a 30% change in REE[7], and another that found a standard deviation of 25%[40], 16 participants are needed in order to allow for a two-tailed alpha level of 0.05 at 80% power.

As stated previously, body composition, specifically FFM, is the strongest predictor of REE. However, most of these studies did not take into account body composition when analyzing their results, and only collected anthropometric measures necessary to screen for BMI.[38,39,6,7] One study used bioelectrical impedance[8], but most studies did not report on anthropometric or body composition measures beyond BMI status.[7,38,39] Recent changes in weight are significantly ($p<0.05$) correlated with changes in RQ,[27,28] and therefore should be accounted for in studies with multiple experimental sessions.

Many of these studies looked for changes in REE after water ingestion based on temperature,[7,38,39] but none of these studies looked for a dose response. All of the studies used either 500 mL[7,38] or 1000 mL of water,[8,39] except the study involving children which used 10 mL/kg of body weight.[6] There did not appear to be a trend between REE and dose based on the outcome of the studies, but none of these studies tested multiple doses in the same subjects.

Last, each study has varying guidelines regarding fluid intake prior to the study. Some specify no water for the full fast,[8] while others omit water for only 1-2 hours prior to the study,[7,39] but no studies took into account hydration status. This study will account for hydration status by assessing urine (**Human Hydration, LLC, Hampton, VA**) and by measuring urine specific gravity (**Fisher Scientific, 13-946-27)**.

Using water as a means to increase sympathetic nervous system activity and REE would provide an inexpensive and feasible means to acutely increase calorie expenditure.

It would provide a safer method for increasing REE and preventing obesity, compared to the many pharmacological interventions that have been pulled from the market due to harmful side effect such as hypertension, cardiac arrhythmias, and worsening of coronary artery disease.[8] The aim of this study to assess whether water could act as a method to prevent obesity.

Outcomes and Hypotheses

Primary Outcome: To examine the relationship between water consumption and resting metabolic rate.

Hypothesis One: There will be a statistically significant ($p<0.05$) increase in REE based on dose response following the ingestion of room temperature water among healthy young adults, and this change will be mediated through activation of the sympathetic nervous system.

Hypothesis Two: There will be a statistically significant ($p<0.05$) shift from carbohydrate oxidation to fat oxidation following the ingestion of water.

CHAPTER THREE: MANUSCRIPT

Introduction

According to NHANES data obesity rates reached 35.5% in 2012 and the prevalence of type 3 obesity (BMI > 40) doubled in only two decades.[1] There is a pressing need for innovative and practical methods to obesity prevention. Research suggests that consumption of water could meet this need through an increase in resting energy expenditure, which makes up between 65-80% of total daily energy expenditure.[13] A method for increasing REE is through activation of the sympathetic nervous system's fight or flight response. When the sympathetic nervous system is activated it further activates the medulla of the adrenal glands, resulting in the release of catecholamines such as epinephrine and norepinephrine.[12] These catecholamines contribute to increased thermogenesis through the regulation of glucose synthesis and use, the mobilization of fat, and protein metabolism.[32] Norepinephrine effect target organs through stimulation of adrenergic receptors.[19] This results in many physiological changes including increased blood pressure,[18] increased production and breakdown of glucose by the liver,[19] lowered insulin secretion by the pancreas, limited uptake of fatty acids in white adipose tissue, and increased uptake of fatty acids by muscle tissue.[19] These metabolic changes play a role in both resting energy expenditure and respiratory quotient.

Research has shown that water consumption can lead to increases in sympathetic activity.[15–17] One proposed mechanism for this increase is through the pressor response. This has been supported by research on subjects with orthostatic hypertension that have shown both an increase in blood pressure and norepinephrine bloods levels after consumption of water.[15] These changes were not replicated during ganglionic blockade, suggesting that these changes were mediated by the sympathetic nervous system. Another explanation for water's role in sympathetic activity is osmolality. A study on baroreflex impaired mice found that only hypo osmotic solutions, not hyper osmotic solutions, led to an increase in blood pressure and these results were prevented after blocking adrenoceptors and the function of norepinephrine.[16] A similar study on human subjects found that only water, not saline solution, led to a significant ($p=0.01$) decrease in heart rate and a significant ($p<0.01$) increase in total peripheral resistance and baroreflex sensitivity ($p=0.01$).

Current literature looking at the role of water consumption in resting energy expenditure is scarce. The research shows mixed results with some studies reporting only minimal changes in resting energy expenditure,[38] while one study claimed to have found a 30% increase in energy expenditure.[7] The same is true of respiratory quotient, with a trend towards a decrease in energy expenditure only in healthy patients[7], and not those that are overweight or obese.[8,39] The purpose of this study is to examine the effects of water consumption on resting metabolic rate.

Methods

Recruitment and Inclusion Criteria

This study took place on the campus of George Mason University, at the department of Nutrition and Food Studies' assessment lab. Advertisements across campus buildings and at on campus fitness centers were displayed for recruitment. The researchers also recruited in person at an on campus fitness center. Following recruitment, subjects were screened either in person or by telephone. Inclusion criteria included an age limit between 18-39 years old. The subjects were both men and women in overall good health, including a BMI within the normal to overweight range ($18.5 \leq$ BMI< 28). Participants had to be able to both write and speak English. This allowed for written consent, and proper communication between the researcher and subject. Subjects had to be willing to eliminate alcohol consumption for 3 days prior to each appointment. They also had to limit their caffeine intake to half of their usual intake three days before each appointment, to a quarter of their usual intake two days before each appointment, and to no caffeine intake the day before each appointment. Subjects were excluded if they were taking medication that affected their fluid balance or metabolism. They also could not participate if they suffered from hepatic, renal, pulmonary, endocrine, or hematological disorders. Dual-energy x-ray absorptiometry (DXA) (**Horizon, Hologic, Bedford, MA**) was used in this study, prohibiting pregnant women from participation. Nursing women were also prohibited, as well as those with claustrophobia due to the requirement to wear an enclosed mask (ventilated hood) while measuring resting metabolic rate. Last, research has shown that changes in weight significantly ($p<0.05$) impacts both RQ and REE,[27–29] therefore participants could not have had more than a 5 kg change in weight during the six months prior to the start of the study.

Protocol

Each participant participated in two experimental assessments. One involved the ingestion of 500mL of room temperature water and the other 250mL. The order in which the volumes were given to each participant was determined by random draw from a bag, in which there was always a 50% chance of receiving 250 mL of water at the first appointment and an equal 50% chance of receiving 500 mL of water. Male participants had a 7-10 day washout period between each appointment. Females also had a 7-10 day washout period, but this was adjusted as needed to ensure that they were assessed during the follicular phase of their menstrual cycle, in order to account for variations in resting metabolic rate across the menstrual cycle.[41,42] Participants were asked to fast overnight beginning at 10pm and arrived at the Assessment Lab between 7:30am and 8:30am. Each participant arrived at the same time for both of their appointments. Informed consent was received upon arrival at the first appointment, followed by a demographic questionnaire.

Anthropometric

Anthropometric measures were taken at the first appointment. Measurement of height was recorded at the first appointment **(Perspective Enterprises, Model PE-AIM-101, Portage, MI**). Waist circumference was measured around the narrowest region of the abdomen using measuring tape. Weight was recorded using a digital scale (**Perspective Enterprises, Healthometer 752KL, Portage, MI**). Weight was measured a second time at the second appointment to ensure no larger than a 1.5 kg change in weight between appointments.

24-hour Dietary Recall

The United States Department of Agriculture's 5-step multiple-pass approach was used to conduct a 24-hour dietary recall at each appointment.[43] This involved the interviewer asking the subject to report on what they had to eat the day before each appointment, using a step-by-step approach. First the subject was asked to list the foods and beverages that they consumed the day before, in no particular order. Next, the researcher probed the participant to see if any foods or beverages were forgotten. During the third step, the time that each food or beverage was consumed was recorded. Next, a detailed description of each food was given including brand, amount, added ingredients, etc. The fifth step involved a final probe for forgotten foods and beverages.[43]

Dual-energy X-ray Absorptiometry

A whole body DXA (**Horizon, Hologic, Bedford, MA**) scan was used to measure fat mass, lean mass and total mass. This involves alternating high and low x-rays which move from the bottom of the scanner, through the subject, and into the machine arm, which scans over the body.[44] The arm has a calibrated disc with a known amount of soft and bone tissue equivalent in it and is calibrated to within 1%. The number of high and low x-rays which are absorbed into this arm after passing through the patient are translated into electronic information on the computer. The result is an image of the body, with the amount of bone mass, fat mass, and lean mass reported for each anatomical region and for the total body.[44] The coefficient of variability for the whole body scan during the study period was 1.1% for fat mass, 1.0% for lean mass, 0.2% for total mass, and 1.1% for percent body fat.

Hydration Status

Each participant was given a urine collection cup prior to both appointments to collect first morning urine. The Urine Color Chart was used to assess urine (**Human Hydration, LLC, Hampton, VA**). Hydration status was also measured by urine specific gravity using a handheld refractometer (**Fisher Scientific, 13-946-27**), which allowed for a quick and precise measure of urine osmolality.[45]

Blood Pressure and Heart Rate

Blood pressure and heart rate were recorded using an upper arm cuff (**Omron Healthcare, Inc., Lake Forest, IL**). Both heart rate and blood pressure were measured at baseline and then every 15 minutes following the consumption of water.

Resting Metabolic Rate

Each participant had their REE measured for 30 minutes using an open circuit metabolic cart (**QuarkRMR, Cosmed, Rome, Italy**). This acted as their baseline REE. After 30 minutes, each participant consumed the given volume of water. They remained seated and the metabolic cart was used to assess change in REE over the next 90 minutes. Data points were recorded every minute for resting energy expenditure and respiratory quotient. Respiratory quotient was measured by taking the ratio between exhaled VCO_2 and Inhaled VO_2. Resting metabolic rate was then calculated using the Weir equation[46]:

Equation 2 Weir Equation: Calculating Resting Metabolic Rate

$$RMR = [3.9(VO_2) + 1.1(VCO_2)]*1.44$$

The first ten minutes was excluded for all participants to allow time for the participants to get comfortable with the metabolic cart and mask placed over their head. The next 20 data points were averaged and used as baseline data. The remaining data points were averaged every 15 minutes and any data outside of two standard deviations was eliminated. Fat and carbohydrate oxidation was calculated based on the volume of inspired oxygen, expired carbon dioxide, and an assumed 10g per 24 hour nitrogen secretion in females and 12g per 24 hours in males using the following equations:[47]

Equation 3 Calculating Carbohydrate and Fat Oxidation

Carbohydrate = 4.55 VCO2 - 3.21 VO2 - 2.87 N

Fat = 1.67 VO2- 1.67 VCO2 - 1.92N

Carbohydrate and Fat Oxidation was recorded every minute. Baseline data was averaged, as well as 15 minute intervals following water consumption. Any minute data points outside of two standard deviations was excluded.

SPSS statistical analysis software was used in the analysis of all data (**IBM, version 23**). Paired sample t-test were used to ensure homogeneity of baseline measures between the two experimental time points. Paired sample t-test were used to assess a dose-response. Analysis of variance and covariance was used to assess the difference between variable means. Linear regression analysis was used to predict the relationship between variables.

Results

Population Characteristics

Based on a similar study that found a 30% change in REE[7], and another that found a standard deviation of 25%,[40] this study aimed to have 16 participants complete the study, allowing for a two-tailed alpha level of 0.05 at 80% power. There was a total of 22 interested contacts, of those 17 met eligibility criteria and participated in the study. One subject was dropped from the study after failing to meet protocol guidelines, and one subject only participated in 1 out of the 2 appointments, both were due to nonadherence to caffeine consumption requirements. This study included data from 16 (M=6, F=10) healthy (BMI: 22.8 ± 1.9 kg/m2) adults (age: 25.9 ± 5.5 years) (**Table 1**). The subjects self-reported their ethnicity, with 12 of the 16 subjects identifying as Caucasian. All participants drank at least 4 cups of water per day on average. The study population was not made up of heavy alcohol or caffeine drinkers, 13 of 16 participants reported that they drank 0-3 alcoholic beverages per week and two or less caffeinated beverages per day. Last, the subjects in this study self-reported that they were physically active.

Table 1 Population Characteristics

	Age	25.9±5.5	Average Cups of Water a Day	
Sex			4-5 cups	43.8%
	Female	62.5%	6-7 cups	6.3%
	Male	37.5%	8 or more cups	50.0%
Anthropometric			Average Caffeinated Beverages a Day	
	Weight (kg)	66.1 ±10.3	0 beverages	12.5%
	Height (cm)	169.0±9.6	1 beverage	25.0%
	BMI (kg/m^2)	22.8±1.9	2 beverages	43.8%
	Percent Body Fat	29.5±5.4	3 beverages	12.5%
	Lean Mass (kg)	45.5±9.4	4 beverages	6.3%
	Waist Circumference	77.7±8.3	Average Alcoholic Beverages a Week	
	Ethnicity		0-3 drinks	81.3%
Caucasian		75%	4-7 drinks	12.5%
	African American	6.3%	No Answer	6.3%

Asian	12.5%	Moderate to Vigorous Activity a Week	
Hispanic	6.3%	0-1 days	12.5%
		2-3 days	37.5%
		4-5 days	31.3%
		6-7 days	18.8%

Homogeneity between Appointments

Paired sample t-test were used to test the homogeneity of variables before each appointment (**Table 2**). Baseline resting energy expenditure was found to be statistically similar between appointments with a mean difference of 47.8 kcal/24hr (±146.6). All dietary variables (total kilocalories, percent kilocalories from carbohydrate, percent kilocalories from fat, percent kilocalories from protein, total fluid intake) were not significantly different. Hydration status between appointments was not statistically similar with a mean urine specific gravity of 1.024 U_{sg} before both appointments (**Table 2**).

Variable	**Mean**	**Std. Deviation**	**Sig.**
Urine Specific Gravity 250 mL	1.024	±. 011	.828
Urine Specific Gravity 500 mL	1.024	±.008	
Baseline REE 250 (kcal/24hr)	1458.3	±262.7	.231
Baseline REE 500 (kcal/24hr)	1505.7	±191.8	
24-Hour Recall			
Total Kilocalories 250 mL	2007.0	±544.5	.165
Total Kilocalories 500 mL	2175.7	±572.5	
% kcal from Carbohydrate 250 mL	54.9	±9.8	.089
% kcal from Carbohydrate 500 mL	50.7	±10.1	
% kcal from Fat 250 mL	31.5	±11.4	.275
% kcal from Fat 500 mL	34.4	±9.4	

% kcal from Protein 250 mL	16.6	±6.0	.624
% kcal from Protein 500 mL	17.3	±5.7	
Fluid Intake (mL) 250 mL	85.9	±24.5	.507
Fluid Intake (mL) 500 mL	81.333	±22.9	

Table 2

Homogeneity Between Appointments

Change in Resting Energy Expenditure

Paired t-tests were used to assess a dose response between 250 mL and 500 mL of water on change in resting energy expenditure (**Table 3**). All 15 minute intervals were compared between the two appointments, there was no found dose response ($p>0.05$). The data was averaged over the 90-minute period, and there was still no significant dose response ($p>0.05$).

Table 3 Dose-dependent Changes in Resting Energy Expenditure (Paired T-test)

Variable	Mean	Std. Deviation	Sig.
Change REE 15 minutes – 250 mL	89.3	±125.1	0.356
Change REE 15 minutes – 500 mL	59.5	±97.3	
Change REE 30 minutes – 250 mL	49.5	±156.4	0.405
Change REE 30 minutes – 500 mL	23.3	±82.7	
Change REE 45 minutes – 250 mL	61.3	±161.1	0.252
Change REE 45 minutes – 500 mL	23.9	±90.3	
Change REE 60 minutes – 250 mL	61.1	±162.5	0.310
Change REE 60 minutes – 500 mL	20.5	±102.0	
Change REE 75 minutes – 250 mL	75.4	±140.9	0.187
Change REE 75 minutes – 500 mL	27.3	±102.1	

Average Change REE over 90 Minutes – 250 mL	69.0	±142.0	0.254
Average Change REE over 90 Minutes – 500 mL	32.6	±88.8	

After no dose response was found, the data from both appointments was combined and time-dependent changes were assessed. ANOVA analysis was used to compare the difference in REE at each 15 minute interval. There was a non-significant ($p>0.05$) trend toward an increase in REE immediately following water ingestion, which remained above baseline throughout the 90-minute period (**Figure 3**). Next a paired t-test was used to compare the average 90 minute change in REE to baseline measures. There was a significant ($p=0.022$) increase in REE from 1472.4 ±229.8 kcal/24hr at baseline to an average 1522.8 ± 188.8 kcal/24hr over the 90-minute period. This was an increase of 50.4 kcal/hr. There was no significant correlation between hydration status and resting energy expenditure.

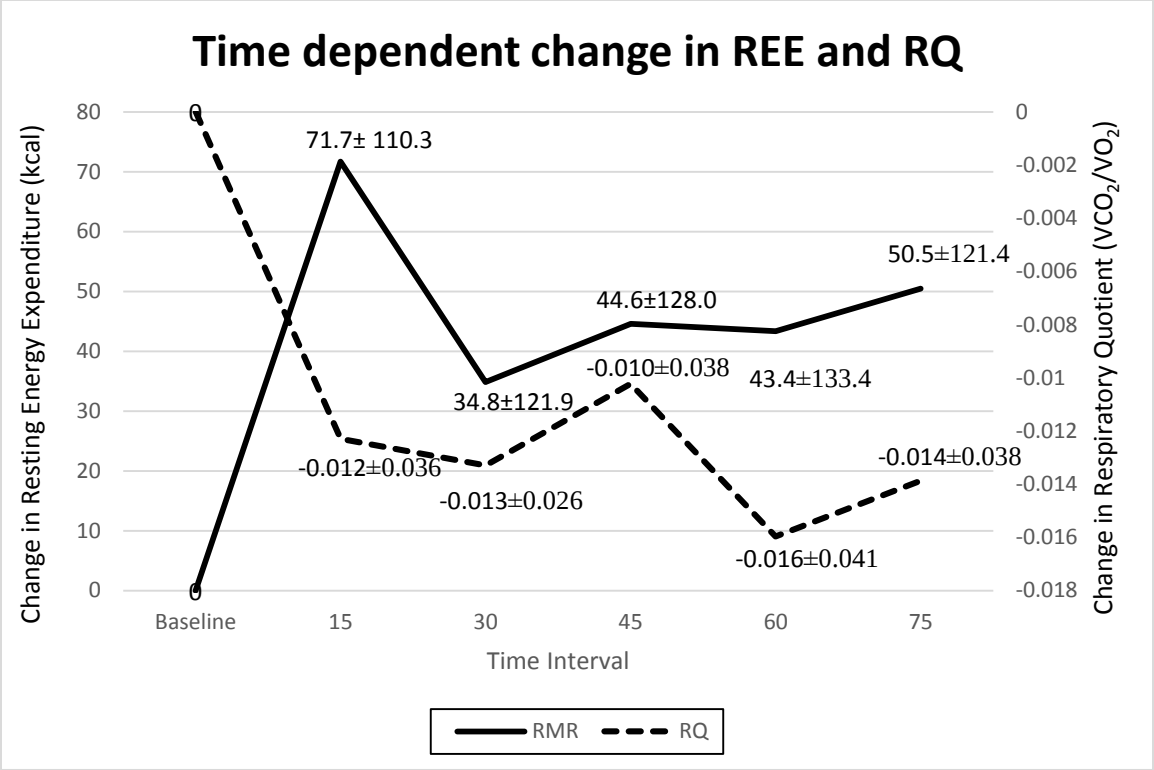

Figure 3 Time Dependent Change in Resting Energy Expenditure and Respiratory Quotient

Change in Respiratory Quotient

Respiratory quotient was assessed in the same manner as REE. First, paired t-test were used to look for a dose response (**Table 4**). There was a statistically significant (p=0.019) dose response found during the 15 minutes following water ingestion. After 250 mL of water there was a 0.003 ± 0.033 increase in RQ compared to a -0.027 ±0.033 decrease after 500 mL of water. No dose response was found at any other intervals or when comparing the average change over the 90-minute period to baseline.

Table 4 Dose-dependent Changes in Respiratory Quotient (Paired T-test)

Variable	Mean	Std. Deviation	Sig.
Change RQ 15 minutes – 250 mL	0.003	±0.033	0.019
Change RQ 15 minutes – 500 mL	-0.027	±0.033	
Change RQ 30 minutes – 250 mL	-0.012	±0.022	0.827
Change RQ 30 minutes – 500 mL	-0.014	±0.031	
Change RQ 45 minutes – 250 mL	-0.001	±0.043	0.072
Change RQ 45 minutes – 500 mL	-0.021	±0.031	
Change RQ 60 minutes – 250 mL	-0.009	±0.049	0.262
Change RQ 60 minutes – 500 mL	-0.023	±0.034	
Change RQ 75 minutes – 250 mL	-0.009	±0.044	0.344
Change RQ 75 minutes – 500 mL	-0.021	±0.031	
Average Change RQ over 90 Minutes – 250 mL	-0.009	±0.027	.221
Average Change RQ over 90 Minutes – 500 mL	-0.020	±0.026	

Data was then combined from both appointments. ANOVA analysis was used to look for a time-dependent change in RQ. There was non-significant ($p>0.05$) time-dependent change in RQ (**Figure 3**). A paired t-test was used to compare the average change in RQ over the 90 minute period to baseline measures. There was a significant ($p=0.005$) decrease in RQ over the 90 minutes from 0.785 ±0.051 at baseline to an average RQ of 0.770 ±0.046 over the 90-minute period. A decrease in respiratory quotient could potentially represent an increase in fat oxidation, the next step in the data analysis was to look for changes in fat oxidation.

Changes in Fat Oxidation

Paired t-tests were used assess if there was a dose-dependent change in fat oxidation (**Table 5**). A dose-dependent change in fat oxidation was found after 15 minutes. The average change in fat oxidation after 250 mL of water was 0.004 g/min (±0.015) compared to 0.015 g/min (±0.013) after 500 mL of water. There were no other significant dose-dependent changes between 15 minute intervals ($p>0.05$).

Table 5 Dose-dependent Changes in Fat Oxidation (Paired T-test)

Variable	Mean	Std. Deviation	Sig.
Change Fat (g/min) 15 minutes – 250 mL	0.004	±0.015	0.043
Change Fat (g/min) 15 minutes – 500 mL	0.015	±0.013	
Change Fat (g/min) 30 minutes – 250 mL	0.006	±0.012	0.724
Change Fat (g/min) 30 minutes – 500 mL	0.007	±0.013	
Change Fat (g/min) 45 minutes – 250 mL	0.005	±0.020	0.258
Change Fat (g/min) 45 minutes – 500 mL	0.010	±0.014	
Change Fat (g/min) 60 minutes – 250 mL	0.008	±0.024	0.553
Change Fat (g/min) 60 minutes – 500 mL	0.011	±0.016	
Change Fat (g/min) 75 minutes – 250 mL	0.008	±0.020	0.497
Change Fat (g/min) 75 minutes – 500 mL	0.011	±0.015	
Average Change Fat (g/min) over 90 Minutes – 250 mL	0.006	±0.016	0.290
Average Change Fat (g/min) over 90 Minutes – 500 mL	0.010	±0.012	

Analysis of covariance showed that dose was a significant ($p=.000$) predictor of the change in fat oxidation over the 90-minute period when controlling for the change in

REE over the 90-minute period (**Table 6**). The estimated mean was greater after 500 mL of water (0.010 ± 0.001 g/min) than 250 mL (.004 ± 0.001 g/min).

After looking for a dose response all data was combined to look for changes in fat oxidation, independent of dose. ANOVA analysis was used to look for time dependent change in fat oxidation, no significant results were found (P>0.05). Baseline data was compared to the average change in fat oxidation over the 90 minutes. A paired t-test showed that there was a significant (p=0.003) increase in fat oxidation from 0.064 g/min (±0.024) at baseline to an average of 0.072 g/min (±0.022) over the 90 minutes following water ingestion. Linear regression analysis indicated that change in REE expenditure over the 90-minute period was an independent predictor of the change in fat oxidation (β=.003, SE=.001], p=.000) (**Table 7**). The change in REE was found to be a significant (p<0.05) predictor of change in fat oxidation at all 15 minute intervals, except after the first 15 minutes, in which dietary fat was a significant (P<0.05) predictor of the change in fat oxidation (**Table 7**).

Analysis of covariance showed many sex-dependent changes in fat oxidation when controlling for co-variables (**Table 6**). A significant effect of sex on fat oxidation over the 90-minute period was found when controlling for the change in carbohydrate oxidation [F(1,181) = 18.715, p= 0.000], homogeneity of variance was not found (p=0.014). Females had a higher estimated mean increase ($\bar{x}$=.009g/min ±.001) than males ($\bar{x}$=.004g/min ±.001). Sex also had a significant effect on change in fat oxidation over the 90-minute period when controlling for percent body fat [F(1,181) = 4.609, p= 0.033]. Females had a higher estimated mean increase ($\bar{x}$=.009g/min ±.002) than males

($\bar{x}$=.003g/min ±.002). The change in fat oxidation after 15 minute was sex-dependent when controlling for total mass (p=.046), lean mass (p=0.44), and waist circumference (p=0.042). The estimated mean was higher in males than females for three all 3 variables (**Table 6**).

Table 6 Analysis of Covariance

Outcome Variable	Predictor Variable	Controlled Variable	DF	Sig.	Estimated Mean (SE)
Δ Fat 15 Minutes	Dose	Δ REE 15 Minutes	(1,28)	.021	250: .004 (.003) 500: .015 (.003)
Δ Fat 15 Minutes	Sex	Total Mass	(1,28)	.046	Female: .001 (.005) Male: .023 (.007)
Δ Fat 15 Minutes	Sex	Lean Mass	(1,28)	.044	Female: .001 (.005) Male: .024 (.007)
Δ Fat 15 Minutes	Sex	Waist Circumference	(1,28)	.042	Female: .004 (.004) Male: .019 (.005)
Average 90 minute Δ Fat	Dose	Average 90 minute Δ REE	(1,181)	.000	250: .004 (.001) 500: .010 (.001)
Average 90 minute Δ Fat	Sex	Average 90 minute Δ Carbohydrate*	(1,181)	.000	Female: .009 (.001) Male: .004 (.001)
Average 90 minute Δ Fat	Sex	Percent Body Fat	(1,181)	.033	Female: .009 (.002) Male: .003 (.002)
*Variable did not meet Levene's Test of Homogeneity of Variance					

Table 7 Predictors of Change in Fat Oxidation (Linear Regression)

Dependent Variable	Predictor Variable	Constant (β)	SE	Sig.
Δ Fat 15 Minutes				
Model 1	Percent Dietary Fat	-0.009	0.008	0.019
Δ Fat 30 Minutes				
Model 1	Δ REE 30 Minutes	0.005	0.002	.000
Δ Fat 45 Minutes				
Model 1	Δ REE 45 Minutes	0.003	0.002	.000
Δ Fat 60 Minutes				
Model 1	Δ REE 45 Minutes	0.004	0.003	.000
Δ Fat 75 Minutes				
Model 1	Δ REE 45 Minutes	0.004	0.003	.000
Average 90 minute Δ Fat				
Model 1	Average 90 minute Δ REE	0.003	0.001	.000

Cardiovascular Response and Change in Resting Metabolic Rate

This study found a non-significant ($p>0.05$) negative trend ($r= -0.269$) between maximum increase in heart rate and maximum increase in resting energy expenditure (**Figure 4**). There was a positive correlation ($r= 0.199$) between the maximum increase in resting energy expenditure and he maximum increase systolic, however this was not statistically significant ($p>0.05$) (**Figure 5**). There was a statistically significant ($p=.009$) positive correlation ($r=0.475$) between the maximum increase in REE and maximum increase in diastolic blood pressure (**Figure 6**). For every 1mm Hg increase in diastolic blood pressure, there is a predicted 9.521 increase in resting energy expenditure. 22.6% of the variance in the maximum increase in resting energy expenditure could be explained by the maximum increase in heart rate.

The relationship between cardiovascular response and respiratory quotient were not as pronounced. There was very small positive trend between maximum increase in heart rate (r= 0.244) (**Figure 7**), systolic blood pressure (r= 0.159) (**Figure 8**) and diastolic blood pressure (r= 0.119) (**Figure 9**) and the maximum decrease in respiratory quotient, however this was statistically non-significant (p>0.05) for all three cardiovascular outcomes.

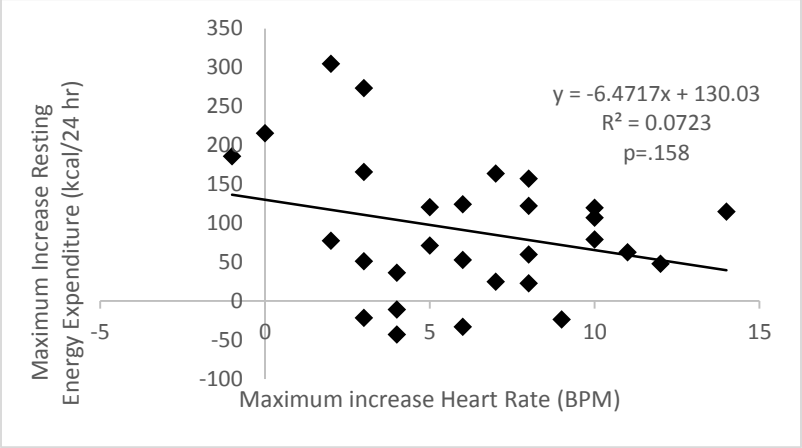

Figure 4 Maximum Increase in Resting Energy Expenditure Predicted by Maximum Increase in Heart Rate

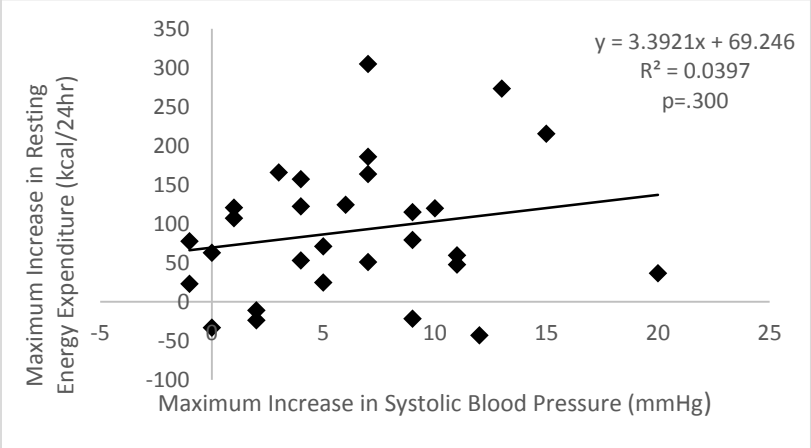

Figure 5 Maximum Increase in Resting Energy Expenditure Predicted by Maximum Increase in Systolic Blood Pressure

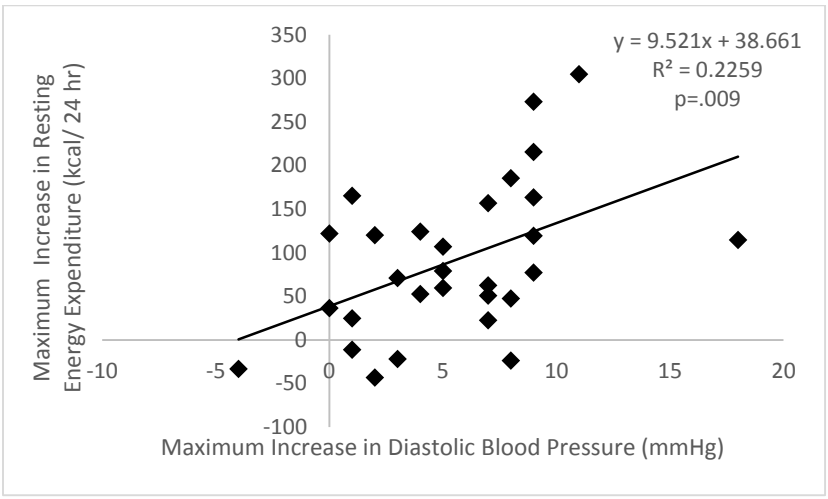

Figure 6 Maximum Increase in Resting Energy Expenditure Predicted by Maximum Increase in Diastolic Blood Pressure

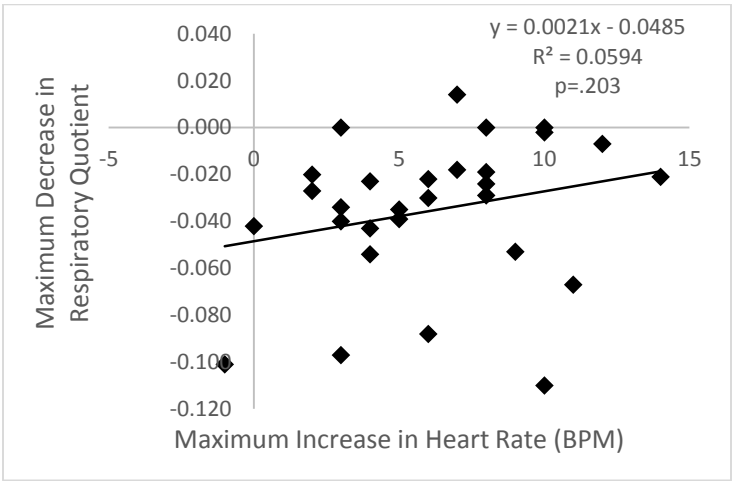

Figure 7 Maximum Decrease in Respiratory Quotient Predicted by Maximum Increase in Heart Rate

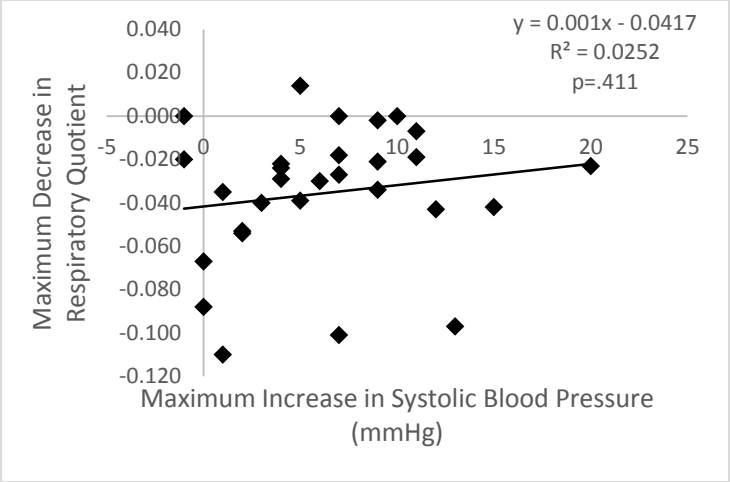

Figure 8 Maximum Decrease in Respiratory Quotient predicted by Maximum Increase in Systolic Blood Pressure

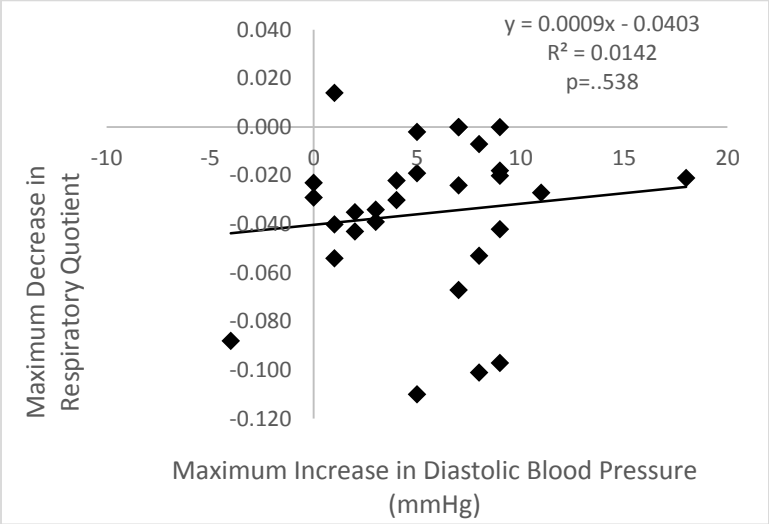

Figure 9 Maximum Decrease in Respiratory Quotient Predicted by Maximum Increase in Diastolic Blood Pressure

Discussion

The purpose of this study was to assess the effects of drinking water on resting metabolic rate in healthy adults. The first hypothesis was that drinking water would significantly increase resting metabolic rate, based on a dose-response, through an

increase in sympathetic activation. This hypothesis was false; no dose response was found. However, after combining the data from both volumes of water, a statistically significant (p=0.022) 3.4% increase in resting energy across the 90-minute period was found. There was also a significant correlation between the maximum increase in resting energy expenditure and the maximum increase in diastolic blood pressure (p=0.009). An increase in blood pressure is an outcome of sympathetic activation and could represent an increase in sympathetic activity. The second hypothesis was that there would be a significant shift from carbohydrate oxidation to fat oxidation. This hypothesis was confirmed with an average of an 11.4% increase in fat oxidation over the 90-minute period.

The findings in this study are in line with many other studies that have found acute increases in resting energy expenditure following water ingestion. A study on 6 participants with similar characteristics as this study found a 4.5% in resting energy expenditure after the consumption of 7.5mL/kg of body weight of 3°C water.[39] Another study found a significant 2.7% increase in REE after the ingestion of 500mL of distilled water, however this change was no longer significant when compared to sham drinking (lifting water to mouth with no ingestion).[48] While many studies have found little to no significant effect of water intake on resting energy expenditure,[38,39,48,49] some have found as high as a 20-30% increase.[6–8,22]. These varying results can be explained by many differences in the methodology used between studies.

One major difference is in the assessment methods used to measure resting metabolic rate. The studies which found the greatest increase used a whole-room

metabolic chamber.[7,22] Another study which found a 20% increase in obese participants and 12% in normal weight subjects did not specify their methods for measuring resting metabolic rate.[8] All other studies, including the current study, used a portable gas analyzer with a ventilated hood.[6,38,39,48,49] All of which did not find greater than a 5% increase in resting metabolic rate, except for one study which was the only study conducted on children.[6]

Anthropometric and body composition characteristics of Subjects

A literary review showed that studies looking at the effects of water on resting metabolic rate include a wide range of BMI's from normal weight to obese. Many of the studies which have found a larger increase in resting energy expenditure have subjects that are overweight or obese,[6,8,22] including one study that found a 20% increase in those that were obese but only 12% in those within a healthy weight range.[8] Whereas, the majority of studies that found only a small increase included subjects within a normal weight range[38,39] based on BMI, or a combination of normal weight and overweight,[48] including the current study. This is an interesting trend because while those studies with obese participants tend to find a larger increase in resting energy expenditure, the opposite is true of respiratory quotient. The same study which found a larger increase (20%) in energy expenditure in those that were obese than those at normal weight (12%), found a significant drop in respiratory quotient only in normal weight subjects.[8] A study on 21 overweight and obese children found a 25% increase in resting energy expenditure, but no significant changes in respiratory quotient.[6] The same was true after finding a 24% increase in overweight and obese adults.[22] An immediate significant drop in respiratory

quotient was found in normal weight adults after both cold water and room temperature water, despite only a 2.9% ($p<0.05$) and non-significant 2.3% increase in resting energy expenditure. The drop in RQ remained significant for 45 minutes following consumption of room temperature water and 90 minutes following cold water.

This previous research suggests that body composition may play a key role in mediating the effects of water ingestion on resting metabolic rate. However, most of the studies do not describe the body composition of their subjects beyond BMI classification, while few utilize bioelectrical impedance to control for body composition.[6,8,48] To the researchers' knowledge, this was the first study to account for body composition using dual x-ray absorptiometry (DXA) (**Horizon, Hologic, Bedford, MA**). A study on subjects with characteristics similar to those in this study, found that DXA was most accurate and least biased in assessing FFM compared to bioelectrical impedance, skin-fold measures, and air displacement plethysmography.[50]

In this study, when accounting for lean mass, there was a significant difference in change in fat oxidation based on sex ($p<.05$), the same was true for percent body fat, total mass, and waist circumference ($p<.05$). When controlling for body composition this study found a much sharper increase in fat oxidation in males, compared to females in the first 15 minutes after water consumption. However, this leveled out when looking at the average 90-minute difference. These results were in contrast to Boschmann et. al. (2003), who found that both men and women had an initial sharp increase in fat oxidation, and then a steady return to baseline after 40 minutes, whereas men continued to have increased fat oxidation after the 90 minutes.[7] However, the found effect of water

consumption on fat oxidation was much larger in their study with a 50% increase in women and 100% increase in men, while our study found an average increase in fat oxidation of 16% in women and 6.1% in men over the 90 minute period. Despite the variation in results of these two studies, they raise awareness to the important role that sex may play in the metabolic response to water and the need for future research in this area.

Dose Response

Varying level of water have been used when looking at the effects of water on resting metabolic rate, however this was the first study to look for a dose response using a crossover design. Our results indicated that there was a dose response ($p=0.019$) in the change of respiratory quotient after 15 minutes. After 250mL of water the average change in RQ was minimal ($0.004 \pm .033$), but after 500mL RQ had a significantly larger change (-0.027 ± 0.032). The change in RQ was accompanied by a significant ($p=0.043$) dose dependent change in fat oxidation at 15 minutes. After 250mL of water the increase in fat oxidation was .004 g/min (± 0.015) compared to a significantly greater increase of .015 g/min (± 0.013) after 500mL of water. Dose was also found to be a significant ($p=0.000$) predictor of the average change in fat oxidation over the 90-minute period, when controlling for change in REE over the 90-minute period. It is possible that large doses of water, such as 1000mL, may elicit a larger impact on resting metabolic rate and fat oxidation. A study using 1000mL found a 20% increase in REE in obese subjects and a 12% increase in normal subjects.[8] While this study did not find a dose dependent change

in resting energy expenditure it is possible that the same study utilizing 500mL and 1000mL of water would find a significant change.

Effect of Temperature and Osmolality on Metabolic Response

A randomized crossover study that compared the cardiovascular and autonomic effects of 500ml of cold (3°C), room temperature (22°C) and body temperature (37°C) water in 12 healthy young adults found that the effects of the water may be dependent on temperature.[38] It found a significant increase in resting energy expenditure only after ingesting 3°C water, not room temperature or body temperature water.[38] Another study used a subset of 4 participants and found that room temperature water resulted in a change of 70kj/hr (16.7kcal), while body temperature resulted in only a 40kj/hr (9.6kcal) change in REE.[7] Similarly, another study found no significant change in REE following room temperature water, but found a significant 4.5% increase in REE after drinking 3°C.[39] It has been postulated that the increase in energy expenditure after drinking water may be due to the thermogenic effect of heating it to body temperature.[22,38,39] In the current study room temperature water was used, it is possible that cold water may have resulted in larger effects.

As stated in chapter one, one proposed mechanism for the increase in resting metabolic after water consumption is osmolality and its effect on the sympathetic nervous system. Research has shown in mice that water ingestion, not saline solution, causes an increase in blood pressure, which is no longer seen after blocking norepinephrine.[16] Similarly, in humans only water, not saline, caused a significant decrease in heart rate and significant increase in peripheral resistance.[15] Neither of these studies measured

resting energy expenditure, but from these studies it can be implied that an increase in REE through sympathetic activation may be dependent on the osmolality of the water, however the results are not clear. Boschmann et al. (2003) has reported the largest increase in resting metabolic rate at 30% using distilled water.[7] The same study found that the change in resting metabolic rate was significantly ($p<.0001$) greater after distilled water than saline solution. The researchers repeated this study a few years later and again found a significant increase in resting metabolic rate, this time a 24% increase.[22] It was also found that venous plasma osmolality significantly decreased after drinking water, highlighting the effects of drinking hypo osmotic solutions. When β-adrenergic receptors were blocked, the changes in metabolic rate were almost completed gone in 6 out of 7 subjects. This study showed that the increase in resting energy expenditure was due to sympathetic activation, which may have been cause by a decrease in plasma osmolality.[22] Similarly, a study using 1000mL of low mineralized sparkling water was found to cause a 20% increase in REE in obese subjects and 12% in normal weight subjects.[8] Despite these change, no significant changes in heart rate or blood pressure were found in either of these studies. Contrary to these studies, other studies have not found significant increases in resting metabolic when using distilled water,[39,48] including no significant differences in comparison to 0.9% saline solution.[39] The differing types of water used in these studies could explain some of the variations in the results.

Cardiovascular Response Outcomes

This study found a negative trend between heart rate and resting energy expenditure, and a positive trend between systolic blood pressure and diastolic blood

pressure and resting energy expenditure. For heart rate and systolic blood pressure only a small, non-significant percent of the variance in resting energy expenditure could be explained. However 22.5% of the variance in resting energy expenditure could be explained by a change in diastolic blood pressure ($p=0.009$). It is possible that the changes in resting energy expenditure were mediated by the sympathetic nervous system, however it would likely be in response to the change in diastolic blood pressure and not heart rate or systolic blood pressure. This is not conclusive from this study or from previous research. Most of the found studies did not report on the cardiovascular response to water ingestion. One study did not find a significant change in resting metabolic rate but did find significant changes in both diastolic and systolic blood pressure,[39] while another which found only a small increase in resting metabolic rate after cold water found a significant decrease in heart rate.[38] The two studies which found the largest increase in resting metabolic rate, reported no changes in cardiovascular outcomes.[8,51]

Hydration Status

This was the first study which accounted for hydration status prior to each appointment. Plasma osmolality is the ideal method to use to assess hydration status, but the required tools are expensive and require blood draw.[45] Urine color and urine specific gravity provided a more feasible alternative. Urine color charts are used often by clinicians to assess hydration status.[10] They are cheap and give immediate results, but are mainly indicators of those that are poorly hydrated or well hydrated and are less effective in detecting small variations. Urine Specific Gravity compares the density of urine to distilled water and will detect smaller variations.[45] Urine concentration is determined by

the number of particles in the urine and the temperature of the urine. USG has been shown to be accurate in determining urine osmolality and hydration status.[52] Our results showed that the urine specific gravity was similar between both appointments (p=.457). The mean for 250mL of water was $1.024U_{sg} \pm .011$ and $1.024U_{sg} \pm .008$, indicating that hydration status between appointments were similar and that the participants were on average hydrated. This was found to be highly correlated with urine color (r=.860, p=.000), improving the credibility of using U_{sg} to assess hydration status. Future studies should ensure that participants are similarly hydrated before appointments.

Implication of Findings

Even though the increase in resting metabolic rate found in this study is small, it should not be discounted. It is possible that continued consumption of water throughout the day could result in a prolonged increase in resting energy expenditure throughout the day. Just an acute increase in energy expenditure by 50 kcal/day extrapolated over a year is equivalent to 5.2 pounds. Using NHANES data and data from the Coronary Artery Risk Development in Young Adults Study, Hill et al. (2003) developed what is known as the energy gap.[53] They looked at the rate of weight gain among 20 to 40 year olds and found that on average there is a 14-16pound weight gain over the previous eight years. Using the data, they estimated the distribution of energy balance, and found the median amount of excess energy taken in each day. It was found to be 15 kcal, and at the 90^{th} percentile was an excess of 50kcal/day. They postulated that 100% of energy is not stored, but rather only about 50%, concluding that an increased expenditure of 100kcal

per day could have prevented weight gain in 90% of the study population.[53] This study raises awareness to the long term implication of short-term changes in energy balance.

The most alarming finding in this study was the 11.4% increase in fat oxidation. Fat oxidation increased from .06362 g/min ± .02449 at baseline to a 90-minute average of .07179 g/min±0.02199. There was an average increase of 0.00818g/min (±.0139). Many studies have shown that a high RQ, and thus low fat oxidation is predictive of future weight gain.[27–29] In a study without intervention, 43 healthy, non-obese women had their resting metabolic rate, respiratory quotient, and anthropometric measures measured at baseline, 3 years, and 6 years.[31] RQ was found to be predictive of weight gain at both the three year and 6 year mark ($p<0.05$). In the 5 participants with the highest RQ (above the 90th percentile) it was an even higher predictor of weight gain ($P<0.001$) with an average increase of 1.5 kg per year in these participants.[31] In a much more controlled study, 111 obese Pima Indians were put on weight maintenance diet for an average of 7 (±4) days.[27] Each participant then spent 24-hours in a metabolic chamber and were followed up after an average of 25 (±11) months. RQ was found to be significantly correlated with both weight gain and body fat mass.[27] An increase in fat oxidation will lower RQ, thus decreasing weight gain. This is especially applicable to those that have metabolic inflexibility, and do not efficiently shift between carbohydrate and fat oxidation in response to dietary intake or during a fasted state.[54]

Conclusion

This study examined the effects of water consumption on resting metabolic rate. It was the first study to control for body composition using a DXA, the first to account for

hydration status, and the first study to look for a dose response in the effects of water on resting metabolic rate. This study found a small, but significant increase in resting energy expenditure and decrease in respiratory quotient. The most novel finding was a significant 11.4% increase in fat oxidation. It also demonstrated that the effects of water on fat oxidation are both dose and sex-dependent. Despite these findings, future research is needed to validate these findings. Among the literature, there is high variability in the outcomes of similar studies. This is likely due to small sample sizes which make it difficult to account for individual variability and differences in methodology. Resting metabolic rate is highly variable both between and within individuals. Larger sample sizes are needed to account for this variability and reach statistical significance. This paper highlights many differences in methodologies including osmolality, temperature, body composition, method of calorimetry, and dose. Future studies should be carefully designed to account for theses variations. Regardless of the methodology, it is evident that the impact of water on resting metabolic rate is mediated through the sympathetic nervous system. However, the mechanisms are unclear and should be addressed in future studies. Overall, it can be concluded that water does have a significant effect on resting metabolic rate, however the extent of this effect is not clear. This study showed that water could provide a cheap and feasible method for increasing fat oxidation, however future research is needed to determine if it is a viable method for increasing resting energy expenditure and aiding in weight maintenance and obesity prevention.

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