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Effect of Water Consumption on Resting Metabolism in Adults

Brittany Leigh Murphy

# A thesis submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of

Master of Science

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#### ABSTRACT

#### Effect of Water Consumption on Resting Metabolism in Adults

Brittany Leigh Murphy Department of Exercise Sciences, BYU Master of Science

This study analyzed the acute effect of water consumption on resting metabolic rate (RMR). It was hypothesized that water would have a small, nonclinically significant effect on RMR.

Men and women ages 18–40 years participated in a crossover study in which each participant received a No Water and Water condition (order determined randomly) with a 7-day washout period between each condition. Both conditions began with visual analog scales to gauge hunger and thirst levels, urine spectrometry to quantify hydration status, and height and weight measurements. The No Water condition consisted of a 30-minute rest period followed by 45 minutes of RMR testing. The Water condition was identical except for the administration of 500 ml of purified water at 3 °C 10 minutes prior to the beginning of the RMR measurement. Resting metabolic rate testing was done via indirect calorimetry.

There was not a condition-by-time difference in 24-hour resting energy expenditure, oxygen consumption, or metabolic equivalents when including all data points and controlling for nonlinearity (ps > 0.0682). There was a significant difference in respiratory quotient (RQ) (F = 13.73; p = 0.0006) with the No Water condition showing a slightly higher RQ than the Water condition. The nonlinear pattern was primarily driven by the first several minutes of testing. Accordingly, we completed analyses without the first 5 minutes of data. The results persisted; that is, there was no condition-by-time effect in 24-hour resting energy expenditure, oxygen consumption, or metabolic equivalents (ps > 0.2435). Further, the RQ remained significantly different (F = 10.57; ps > 0.0023); however, it was slightly higher in the Water condition.

This study did not support our hypothesis that consumption of 500 ml of water would have a measurable effect on RMR and fuel utilization compared to not consuming water. Rather, this study replicates other studies that suggest there is not an acute measurable effect of water consumption on RMR. Nevertheless, one positive application of these findings is that water may be a suitable control in RMR studies. In addition, these results should not discourage overall water consumption for healthy functioning. Further, consumption of water-rich foods over time could be an effective strategy for weight management (as shown in other studies). Future studies could attempt to determine if larger volumes of water or different temperatures of water have an effect on RMR.

Keywords: resting metabolic rate, RMR, effect of water, water, metabolism, resting energy expenditure, REE, metabolic rate, fluids, energy expenditure

# ACKNOWLEDGEMENTS

Thank you to my husband for all of his love and support through this process. Thank you to my family for the same. Thank you to my many research assistants for their assistance and hard work. Thank you to my chair and committee for their kindness, help, and service.

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#### Introduction

Obesity is one of the most significant public health problems faced by the developed world. In the United States, 39.8% of the adult population live with obesity (Hales et al., 2017). Obesity is defined as a body mass index (BMI) of greater than or equal to 30 kg/m<sup>2</sup>. Obesity has been linked to multiple chronic diseases, such as diabetes mellitus, cardiovascular disease, various forms of cancer, and cerebrovascular disease (Corona et al., 2015). These are among the leading causes of mortality in the United States (Corona et al., 2015). Recently, joining with other organizations, the American Medical Association designated obesity as a disease: thus, demonstrating the importance of obesity treatment and prevention (Ata et al., 2017). While not as pronounced as obesity and debatable, overweight (BMI 25–29.99 kg/m<sup>2</sup>) may also increase risk of some diseases (Corona et al., 2015).

Weight change is a function of energy balance or the balance of energy intake and energy expenditure. Though its influences are multifaceted and complex, energy intake may be simply described as the amount of energy consumed in food or beverages and expressed as kcal/day. On the other side of the energy balance equation is energy expenditure. Total energy expenditure (TEE) is the sum of resting metabolic rate (RMR), thermic effect of food (TEF), and the energy expenditure of all physical activity (Segal, 1984). RMR is a measure of the energy cost while in a state of wakefulness without performing physical activity (Ravussin & Bogardus, 1992). The TEF describes the energy cost of digestion and metabolism of nutrients from food as well as the heat lost during these processes (Ravussin & Bogardus, 1992). Energy expenditure via physical activity is highly variable depending on multiple factors including individual fitness, intensity and duration of an activity, climate, etc. (Ravussin & Bogardus, 1992). Intentional exercise is a subset of physical activity (Ravussin & Bogardus, 1992).

Increasing physical activity is often considered to be the key to increasing TEE as people can "burn off" the excess kcals consumed and because it is the most modifiable component of energy expenditure (Cornier et al., 2017). In actuality, physical activity accounts for only 17– 32% of TEE, while RMR accounts for 60–75% and TEF for the remaining ~8% (Segal, 1984).

Many public health programs addressing overweight or obesity have focused on increasing physical activity, yet this has not curbed national prevalence trends (Maraki et al., 2005). Unfortunately, even when a program is successful in increasing activity level, nearly 50% of all participants in the program will stop within six months (Maraki et al., 2005). Furthermore, ~60% of adults with obesity report trying to lose weight with exercise; however, less than 20% of adults with obesity meet physical activity recommendations from the American College of Sports Medicine (ACSM) (Donnelly et al., 2009). More than 250 minutes per week of moderate-intensity activity is recommended for those seeking weight loss (Donnelly et al., 2009). The ACSM also suggested that a calorie restrictive diet be used simultaneously (Donnelly et al., 2009). Clearly, physical activity and exercise have limitations as a primary treatment for obesity.

Maintenance of or increasing RMR may be important over time for weight management. Research has shown the efficacy of stimulants such as caffeine, ephedrine, phentermine and illegal substances to increase RMR (Hainer & Aldhoon-Hainerova, 2014). However, these medications and substances may have significant side effects, including cardiovascular damage (Hainer & Aldhoon-Hainerova, 2014). Safer but less effective medications have been brought to market that act mechanistically to decrease energy intake without changing energy expenditure (Hainer & Aldhoon-Hainerova, 2014). Other research has examined strength training, interval training, or training at altitude to increase muscle mass and RMR (Pratley et al., 1994; Schubert et al., 2017; Woods et al., 2017). Though increasing muscle mass effectively raises RMR, it is difficult and takes time. In addition, each additional kg of muscle results in an increase of ~13 kcal/day; thus, only modestly improving RMR (Elia, 1992). A safe, effective, and immediate method of increasing RMR remains elusive.

As an alternative, water consumption may influence both energy intake and energy expenditure. Previous evidence suggests that sufficient consumption of foods low in energydensity, which are also high in water content, can reduce weight or prevent weight gain (Dennis, 2010; Stookey, 2008). Interestingly, multiple studies have reported reduced kcal consumption without changing subjectively reported fullness in participants consuming low energy dense foods compared to those consuming high energy dense foods (Bell et al., 1998; Duncan et al., 1983; Rolls et al., 1999). A study by Rolls et al. demonstrated that participants felt more full after eating soup than a casserole made from the identical ingredients except the additional water in the soup (Rolls et al., 1999).

There is a small body of literature that suggests that direct consumption of water is associated with weight loss. In one study, the combination of increased water consumption and a hypocaloric diet resulted in a ~2 kg greater weight loss compared with participants simply following the hypocaloric diet (Dennis, 2010; Stookey, 2008). Some studies suggest that direct consumption of water may also increase RMR, which could influence energy balance and weight management (Stookey, 2008). Specifically, several studies have reported that increased water consumption of 400–1000 ml/day results in a 3–30% increase in RMR (Boschmann et al., 2003; Boschmann et al., 2007; Dubnov-Raz, 2011; Kocelak et al., 2012). This increase appears to peak approximately 45 minutes post-water-consumption and remains elevated for 90+ minutes post-water-consumption (Boschmann et al., 2003; Boschmann et al., 2007; Dubnov-Raz, 2011;

Kocelak et al., 2012). On the other hand, several studies have reported no change in RMR with water consumption (Brown et al., 2006; Charrière et al., 2015).

Water temperature appears to account for a significant portion of the changes in RMR post-water-intake reported by Boschmann et al. (2007). Boschmann found that 30–40% of the increase in RMR was due to raising the water temperature from room temperature (22 °C) to body temperature (37 °C). This closely approximates the known energy requirements of raising water 15 °C, or about 7.2 kcal per 500 ml. Brown et al. hypothesized that drinking cold water (3 °C) would result in an even higher change in RMR due to the greater temperature difference between the water and body tissues (Brown et al., 2006). They found the water increased RMR by 3.6 kcal over 90 minutes, much less than the predicted 16.7 kcal required to heat the water to body temperature (Brown et al., 2006). They predict that the body raises the water temperature not by increasing RMR, but by controlling for heat loss via vasoconstriction (Brown et al., 2006). Kocelak et al. found an increase of 3.6 kcal per 1000 mL of room temperature (22 °C) water (Kocelak et al., 2012). Girona et al. found that chilled and/or room temperature water had an effect on hemodynamics, but that hemodynamics were unaffected by consumption of body temperature water (Girona et al., 2014).

These mixed results and the potential positive application of water consumption for energy expenditure and weight management beg several questions: 1) does water consumption indeed increase RMR, and if so, 2) are the changes clinically significant for weight management?

In short, direct water intake (not from water-rich foods) may influence energy expenditure through RMR though the data are not unequivocal. Unfortunately, the available literature is sparse and mixed; even worse, it tends to be poorly powered and with few studies using participants with excess weight. Additional research is needed in this area to better

understand the relationship between water consumption and RMR and its implications for weight management. Therefore, we proposed the following study.

# **Statement of Purpose**

Our purpose was to determine the effect of water intake on RMR over 45 minutes postconsumption in a sample of adults primarily with overweight or obesity. Purified water was used to make results most generalizable.

We hypothesized that water intake would significantly increase RMR compared to not consuming water, but the results would be clinically modest.

### Methods

### **Study Design**

This study was approved by the Institutional Review Board at Brigham Young University. In addition, the study design, power and statistical analyses were posted on the Open Science Framework prior to initiation of the study. This study used a crossover design, with two experimental sessions, and the order was determined randomly (see Figure 1). There was one short administrative session prior to the experimental sessions. Participants were then scheduled for two ~90-minute sessions separated by seven days, with the second visit taking place on the same day of the following week. They were asked to schedule their administrative visit 1–2 days prior to their first experimental session. The 1-week period between experimental sessions allowed for a wash out period and controlled for possible weekday variation that could influence results. The No Water session consisted of 30 minutes of rest (in preparation for the RMR test), followed by a 45-minute RMR test using indirect calorimetry via metabolic cart. Metabolic rate was measured continuously during the 45-minute RMR test. The Water session was identical, except for the oral consumption of 500 mL of purified water (Kirkland brand, Kirkland, WA) at 3 °C 10 minutes before the initiation of the RMR test.

#### **Population**

Conclusions apply to men and women between the ages of 18 and 40 years of age and with a BMI primarily in the overweight range.

## Sample/Participants

Participants were recruited from the BYU community and the areas in and surrounding Utah County. Participants were men and women between the ages of 18 and 40 years and selfreported a body mass index (BMI) of at least 25 kg/m<sup>2</sup>. Individuals were excluded if they selfreported a BMI less than 25 kg/m<sup>2</sup> or more than 40 kg/m<sup>2</sup> and who were pregnant or lactating. Other exclusion criteria include: taking prescribed or over the counter medications affecting fluid balance or metabolism (i.e., diuretics, laxatives, antacids, anti-histamines, or blood pressure medications (Jawad, 2017)), diagnoses of hepatic, renal, pulmonary or hematological disease, eating disorders, performing more than three hours of strenuous exercise per week, consuming more than two alcoholic beverages per day on average, more than five pounds of weight change in the prior six months, and/or those with claustrophobia. Participants had to be willing and able to fast for eight hours before each appointment and avoid caffeine, smoking, and strenuous exercise in the 24 hours preceding each appointment. Participants who did not comply with these restrictions were rescheduled for another day or excluded from the study. Participants were screened via email for these issues in addition to general health risks and answers were confirmed in person at the administrative visit.

# **Independent Variables**

Water condition (No Water; Water): Oral consumption of 500 ml of purified water at 3 °C.

Time: 45 minutes of resting metabolic rate.

#### **Dependent Variables**

Resting metabolic rate and fuel utilization (carbohydrate and fat) of participants.

# Procedures

The first session was administrative in nature, with the participant coming in at any time of day. This session consisted of explaining the procedure and reviewing the informed consent document. A member of the research team explained the risks, benefits, and details of the study. After addressing any questions, each participant was invited to sign the informed consent document. The participant then received a urine collection cup and a 500 ml bottle of water (Kirkland brand). They were instructed to drink the bottle of water the evening before their first experimental session, beginning after dinner and finishing before going to sleep. This was to increase the likelihood that all participants were in a state of euhydration. They were then instructed to collect a sample of their first urine in the morning before attending their first experimental session and bring the sample cup with them.

Prior to each experimental session (No Water; Water conditions), each participant refrained from strenuous exercise, smoking, and caffeine for 24 hours. They also abstained from food and water for at least 8 hours (no more than 14 hours) preceding each session and slept at least 7 hours the night before each session. Each participant drank their bottle of water after dinner and before going to bed and collected their first urine in the morning.

Participants were sent an email reminding them of these requirements 24 hours before their appointment. The morning of their appointment they were asked about these requirements and were rescheduled if they did not comply.

Participants attended both experimental sessions between the hours of 6:30 am and 9:00 am. Upon arrival at the laboratory, they were asked to give the researcher their urine sample and to complete several questionnaires and a visual analog scale assessing hunger and thirst level. Subsequently, each participant moved to a private dressing room and changed into a standard hospital gown and removed their socks and shoes. While the participant was changing, the researcher conducted the urinalysis with a handheld refractometer. The specific gravity of the urine sample was recorded. The participant was measured for height using a wall-mountedstadiometer and weighed using a digital scale. Each participant was instructed on how to put a POLAR heart rate monitor on their chest and then went back to the dressing room to re-dress and put on the heart rate monitor in private. The participant then began their No Water or Water condition based on random assignment.

#### Experiment

#### No Water Condition

As seen in Figure 2, once randomized to the No Water condition, each participant began by resting on an exam table in a reclined seated position for 30 minutes (run-in period). Heart rate was monitored throughout the rest period. After 30 minutes, the exam table was lowered to allow the participant to lie flat in a supine position. The RMR measurement was initiated by placing a ventilated hood (canopy) connected to a metabolic cart (indirect calorimetry) over each participant.

After 45 minutes of RMR measurement, the canopy was removed, and the participant was asked to slowly sit up and readjust to movement. The participant then removed the heart rate monitor. If this was the first visit, the date and time for the next session was confirmed and a new urine collection cup and water bottle were issued. If it was the second visit, each participant was thanked for their time and participation in the study, compensated, and dismissed.

#### Water Condition

As seen in Figure 2, once randomized to the Water condition, the research protocol was identical to the No Water condition except that each participant consumed water 10 minutes before the RMR test. Thus, each participant was asked to rest on the exam table in a reclined seated position after arrival at the laboratory. After 20 minutes, the participant was asked to drink 500 mL of purified water (Kirkland brand; 3 °C) through a straw while remaining reclined and was given three minutes to consume the water in full. The purified water (Kirkland brand) was chosen for generalizability and the 3 °C temperature was chosen to be consistent with the average refrigerator temperature recommended by the U.S. Food and Drug Administration (FDA) (Center for Food Safety and Nutrition, 2017).

After consuming the water, the procedures for measuring RMR were identical to the procedure noted above for the No Water condition. We note, based on previous studies (Boschmann et al., 2003; Boschmann et al., 2007; Dubnov-Raz, 2011), the effect of water on metabolic rate peaks between 40–45 minutes; therefore, if water influenced metabolic rate, the peak could be captured with the RMR measurement duration and protocol of this study (Boschmann et al., 2003).

# Measurements

# **Demographic Information**

Using a standard form, each participant was asked to provide information about their demographics and lifestyle. These data were used to include/exclude potential participants based on criteria for the study and to describe the sample of the study. In addition to questions to assess exclusion/inclusion criteria, data collected included: age, sex, race, ethnicity, years of education, self-reported height and weight, weight history, marriage status, whether or not pregnant or lactating (for female participants), family history, use of alcohol, tobacco, and recreational drugs, history of eating disorders, and medication use.

# Visual Analog Scale

A visual analog scale measuring hunger and thirst level was given. A visual analog scale (VAS) is composed of a question, such as, "How hungry do you feel right now?" paired with a 10 cm line with statements at each end, representing a continuum from "not at all" to "extremely." Participants marked a vertical line representing their subjective feeling at that moment. A research assistant measured the distance from the left end of the line to the vertical mark with a standard ruler to the nearest 0.5 mm. A VAS is considered a valid and reliable measure of subjective feelings, avoiding bias imposed by categorical response types (Blundell et al., 2010).

#### Height and Weight

After voiding, each participant was measured for body weight to the nearest 0.01 (kg) using an OHAUS Defender 5000 digital scale (OHAUS Corporation, Parsippany, NJ, USA) and with each participant in bare feet and a hospital gown. Next, height (cm) was measured using a

wall-mounted SECA 216 stadiometer (seca north america, Chino, CA, USA) also with each participant in bare feet standing at full extension with the eye-line parallel to the floor. *Urinalysis* 

Each participant collected their first urine at home during the morning of their experimental session. Each participant brought a urine sample, collected in a 4 oz specimen cup (Healthstar, Amazon.com) to the lab (see Procedures), where it was tested using a handheld refractometer (Hand-Held Refractometer, Atago USA, Inc, Bellevue, WA). Refractometry uses a measurement of the urine's refractive index as an indirect estimation of specific gravity (Chadha et al., 2001). The refractive index is the ratio of the velocity of the light in air to the velocity of light in solution; the difference in velocity causes deviation in the path of light (Chadha et al., 2001). The number and type of solutes in the urine is proportional to the degree of refraction (Chadha et al., 2001). A measurement outside of the normal range (>1.020 g/mL) indicates dehydration (Zubac et al., 2018).

Calibration for handheld refractometers is simple: a drop of distilled water is placed on the refractometer window and viewed to determine that the refractometer still reads 1.000 g/mL. To measure the specific gravity of urine, a drop of urine is pipetted onto the refractometer reference glass window (Chadha et al., 2001). The refractometer's cover-plate is carefully dropped, creating a thin film of urine covering the glass (Chadha et al., 2001). Upon looking through the refractometer's eyepiece and holding it up to the light, a researcher can observe the specific gravity on the internal scale (Chadha et al., 2001). Handheld refractometers are a valid and reliable method for determining specific gravity of urine (Armstrong et al., 1998; Minton et al., 2015).

# Indirect Calorimetry

Indirect calorimetry was used to determine energy expenditure (EE) and respiratory quotient. Indirect calorimetry is a valid method for determining EE and respiratory quotient RQ) (Cooper et al., 2009; Bassett et al., 2001; Welch et al., 2015). Parvo Medics TrueOne 2400 was used with the canopy system to capture RMR. The Parvo Medics TrueOne 2400 system is considered valid and reliable (Cooper et al., 2009; Welch et al., 2015). The Parvo Medics measures the fraction of expired oxygen (FEO<sub>RQ</sub>), fraction of expired carbon dioxide (FECO<sub>2</sub>) and volume of expired air (VE) in order to determine the volume of carbon dioxide produced (VCO<sub>2</sub>), RQ, volume of oxygen expired (VO<sub>2</sub>), and kilocalories per day (Welch et al., 2015). The rate of CO<sub>2</sub> production indicates the rate of metabolism of O<sub>2</sub>, thus the overall rate of O<sub>2</sub> metabolism required to support the body in a state of rest (Bassett et al., 2001). The ratio of VCO<sub>2</sub>/VO<sub>2</sub> is called the respiratory exchange ratio (RER) and estimates the RQ, which is an indicator of fuel utilization in the body (Bassett et al., 2001; Widmaier et al., 2016). A value of 0.7 indicates that all substrate metabolized are lipids; a value of 1.0 indicates entirely carbohydrate sources, at an assumed steady state (Hinkle, 2017; Widmaier et al., 2016).

Calibration Procedures for the RMR Parvo Medics TrueOne 2400 include a warm-up period of 30 minutes, followed by flowmeter calibration via a 3 L syringe to confirm accurate measure of airflow during testing. Gas calibration is also performed, measuring a reference gas (1.004% CO<sub>2</sub>, 16.00% O<sub>2</sub>, balance N<sub>2</sub>) and comparing O<sub>2</sub>/CO<sub>2</sub> measurement results to the known composition of the reference gas in order to confirm accurate gas analysis during testing.

RMR measurement requires 30 minutes of rest prior to testing to ensure the participant entered a rest state. The participant's study ID and anthropometric data were entered into the program along with current temperature, barometric pressure and humidity to ensure accurate

testing. The calorimetry hood was connected to the Parvo Medics and placed over the participant's head and upper body, with the edges of the connected plastic sheet tucked under the participant to ensure a seal. When CO<sub>2</sub> numbers stabilized, this indicated the test could begin. The target CO<sub>2</sub> percent was 1.00%, but between 0.80–1.20% was acceptable and could be adjusted via the flowmeter. CO<sub>2</sub> percent was monitored throughout the test. After 45 minutes, the hood was removed, and the participant prepared to leave. The Parvo Medics was turned off to conserve the life of the machine.

#### Heart Rate

Baseline heart rate was measured throughout both experimental sessions. Heart rates from minutes 10–20 were averaged to determine resting heart rate. Heart rate was recorded throughout the RMR tests in order to make note of any large changes in heart rate that may explain any changes to RMR unrelated to water intake, such as movement, startles, falling asleep, etc. POLAR heart rate monitors (Polar Electro; Kempele, Finland) were used to record heart rate throughout each session. POLAR heart rate monitors are widely considered valid and reliable (García-González et al., 2016).

#### **Sample Size Calculation and Power Analysis**

A priori sample size determination for this study (G\*Power, version 3.1.9.2) was based on a small effect size (Cohen's D = 0.25; f = 0.125) for water consumption on RMR and 90% power. Previous studies have reported a relatively small sample sizes (n = 8–45). We note that the study with n = 45 participants analyzed two groups separately; thus, actual sample sizes range from n = 8–24. Using statistically significant studies, previous studies show effect sizes (Cohen's D) ranging from 0.28 to >2.0 (mean = 1.09). Therefore, utilizing a small effect size of 0.25 seemed reasonable and conservative. Using an F test of repeated measures and withinbetween factors (two conditions and repeated 1-minute metabolic measurements during rest), an alpha level of 0.025, an effect size (f) of 0.125, and moderate correlation among measures (0.5), 42 participants (tested twice) were needed to achieve >90% power. This is nearly double the sample size of the largest previously available study and as much as six times the sample size of other studies.

## **Statistical Analysis**

The statistical software SAS, version 9.4 (Cary, NC), was utilized for analyses of the data. During the RMR measurement periods (45 minutes) for both the No Water and Water conditions, metabolic rate was averaged in 1-minute increments and analyzed across the 45 minutes. Hierarchical linear modeling was used to compare changes in the No Water and Water conditions over the duration of the RMR tests. Accordingly, the mixed model procedure in SAS was employed to test for a condition-by-time interaction with time and participant as random variables. In addition, to account for a nonlinear pattern, the square of time was added to statistical models. We further show several exploratory models where hydration level, age, sex, and/or body weight were included as control variables (see Tables 3 and 4) And with the first 5 minutes of data removed (Table 4). As noted in the Results, several participants self-reported BMIs within the inclusion criteria of the study and were allowed to participate. However, after initiating the study, the measured BMIs of six participants were below 25 kg/m<sup>2</sup> and one participant above 40 kg/m<sup>2</sup>. We included analyses both with and without these participants. The alpha level was p < 0.025 to evaluate statistical significance and correct for multiple comparisons (RMR).

#### Results

Two hundred and seventy-two adults initially expressed interest in our study. Two hundred and twenty-four participants were excluded for self-reporting that they did not meet the study criteria; forty-eight reported meeting the study criteria and were scheduled to participate in the study. Forty-two participants completed all study requirements (~87.5% completion rate) and an additional participant completed the study partially (data included in this analysis). Of these 43 participants, 23 (~54.8%) were randomized to start with the No Water condition, while 20 (~46.5%) were randomized to start with the Water condition. Twenty participants were male (~46.5%) and 23 were female (~53.5%); 37 of the participants were Caucasian (~86.0%), 2 were Hispanic (~4.7%), 2 were Asian American (~4.7%), 1 was Polynesian (~2.3%), and 1 was Native American (~2.3%).

While all participants initially self-reported a BMI of at least 25 kg/m<sup>2</sup> and were allowed to participate in the study, our lab measurements showed subsequently that six of these participants had a BMI < 25 kg/m<sup>2</sup> and one participant had a BMI > 40 kg/m<sup>2</sup>. Nevertheless, these are included in this analysis. Therefore, in the final sample (n = 43), 6 participants were in the normal-weight category (BMI < 25 k/m<sup>2</sup>), 28 participants were in the overweight category (BMI 25–29.99 kg/m<sup>2</sup>), and 9 participants were in the obese category (BMI  $\ge$  30 kg/m<sup>2</sup>); including 1 participant with a BMI > 40 kg/m<sup>2</sup>. On average, participants were 27.98 ± 7.06 years and with a BMI of 28.20 ± 4.59 kg/m<sup>2</sup> (see Table 1).

As shown in Table 2, on average participants arrived at the laboratory with a low-tomoderate level of hunger and moderate level of thirst at the beginning of each laboratory condition (No Water; Water). On average, participants reported a specific gravity that bordered dehydration though numbers were within normal kidney function range. Resting heart rate was within a normal range. There were no statistical differences for variables related to hunger or thirst, hydration, or resting heart rate by condition (ps > 0.39) suggesting consistency between conditions for these variables.

The primary outcome of interest for this study was resting energy expenditure across the duration of the 45-minute tests. With inclusion of all time points over 45 minutes, there was an apparent visual nonlinear pattern; primarily resulting from variability during the first several minutes of the test (see Figure 3). Therefore, data were analyzed with and without controlling for nonlinearity (see Table 3). As shown in Table 3, there was no condition-by-time interaction for 24-hour resting energy expenditure (F = 3.49; p = 0.0688). Oxygen consumption (ml/kg/min) and metabolic equivalents also revealed that there was no condition-by-time interaction (F = 0.68; p = 0.4129). However, there was a significant difference in RQ (F = 13.73; p = 0.0006) with the No Water condition showing a slightly higher RQ than the Water condition. These results were not changed with the addition of multiple control variables in several models. In addition, when including only participants with BMIs between 25–40 kg/m<sup>2</sup> (n = 36) the trends were nearly identical.

The nonlinear pattern was primarily driven by the first several minutes of testing. This is typical in resting metabolic studies where there is some adjustment to wearing the canopy. Accordingly, we completed analyses without the first 5 minutes of data (Fullmer et al., 2015). With and without adjustment for linearity, the results remained similar. That is, there was no condition-by-time difference in 24-hour resting energy expenditure, oxygen consumption, or metabolic equivalents. Further, the RQ remained significantly different; however, it was slightly higher in the Water condition (see Table 4).

As exploratory analyses (Table 4), we further analyzed resting energy expenditure for a condition-by-time interaction (assuming linearity) with control of hydration level, age, sex, and/or body weight in two different models. These adjustments did not influence the results. Nevertheless, there was variance in resting energy expenditure across time. In Figure 3, there appears to be a small sustained increase in resting energy expenditure over minutes 10–20; however, this did not meet our a priori alpha level of p < 0.025 (ps > 0.056).

#### Discussion

The aim of this study was to determine the effect of water intake on RMR and fuel utilization over 45 minutes postconsumption. We hypothesized that water intake would result in a statistically significant increase in RMR compared to not consuming water, but that the results would be clinically modest. After analyzing our data, we found no difference across time between water conditions for oxygen consumption, METs, or resting energy expenditure. The RQ was statistically different by condition across time but was not clinically meaningful, i.e., the difference in RQ does not indicate a significant change in the substrates metabolized. Analyzing the data to account for several variables, and with or without the first 5 minutes of data, did not influence the results. Thus, our hypothesis was incorrect. According to our study, water intake did not influence RMR.

Our findings agree with several other studies which found no acute relationship between water consumption and RMR. In a study by Charriere et al., participants drank 500 ml of 21–22 °C distilled water or performed sham drinking (Charrière, 2015). Although water ingestion increased energy expenditure more than sham drinking, the differences were not statistically significant (Charrière, 2015). Brown et al. reported no effect from 7.5 ml/kg (mean = 518 ml) of distilled water or 0.9% saline; however, a 7% sucrose solution had a statistically significant

effect on RMR (Brown et al., 2006). Interestingly, Brown et al. used sham drinking and found that the coefficient of variation was very similar between actual drinking (3%) and sham drinking (2.3%)—a nonstatistically significant difference (Brown et al., 2006). Brown was attempting to replicate a study by Boschmann et al. and was unable to do so (Brown et al., 2006).

Contrary to our results, several studies have suggested that water does acutely increase RMR. Suggested mechanisms for this include: water temperature and warming of the water (Boschmann et al., 2003; Brown et al., 2006; Girona et al., 2014; Kocelak et al., 2012), osmolality differences (Dubnov-Raz, 2011), cardiovascular-sympathetic response (Brown et al., 2006; Charrière, 2015; Dubnov-Raz, 2011; Jordan, 2005), rehydration of dehydrated tissue improving function (Dubnov-Raz, 2011), and stomach distension (Charrière et al., 2015; Dubnov-Raz, 2011; Girona et al., 2014).

Boschmann et al. report that in both men and women there was a 30% increase in energy expenditure, beginning 10 minutes after consuming water and reaching a maximum at 30–40 minutes after water consumption (Boschmann et al., 2003). The 500 mL of water had a greater effect than 250 mL and participants' hydration status was not significant (Boschmann et al., 2003). Upon revisiting his initial findings and attempting to replicate them in response to Brown et al.'s failure to do so, Boschmann et al. found an increased RMR of 24% (Boschmann et al., 2007). In addition to the replication of his previous study, Boschmann et al. had his second round of participants consume 500 ml of water, 50 ml of water, or 500 ml of saline to assess possible mechanisms (Boschmann et al., 2007). Neither 50 ml water nor 500 ml saline had a significant effect on resting energy expenditure (Boschmann et al., 2007). Boschmann et al. used a whole-room calorimeter for both studies; to our knowledge no other researchers have followed this protocol (Boschmann et al., 2007; Boschmann et al., 2003; Brown et al., 2006). To our

knowledge, Boschmann et al. results have not been replicated by other studies (Brown et al., 2006).

With a more similar sample population to our study, Kocelak et al. found a 12% increase in energy expenditure in lean women and a 20% increase in EE in women with obesity after the ingestion of 1000 ml of low mineralized nonsparkling water at 22 °C (Kocelak et al., 2012). Kocelak et al.'s use of 1000 ml of water (twice the amount used in our study) likely influenced the results due to the high gastric load created (Dubnov-Raz, 2011; Kocelak et al., 2012).

We note the difference in study designs between our study and others. For example, other studies tended to have small sample sizes [e.g., n = 14, n = 16, n = 45 (21 normal-weight and 24 with obesity] (Boschmann et al., 2007; Boschmann et al., 2003; Kocelak et al., 2012). Our study was well-powered with 43 participants (n = 37 with a BMI of at least 25 kg/m<sup>2</sup>), and we utilized a crossover design with each participant completing two separate RMR tests (No Water and Water). Additionally, there is variety in the length of time of the RMR tests. Kocelak et al. examined research subjects for 90 minutes (30 minutes of baseline RMR plus 60 minutes postwater-consumption (Kocelak et al., 2012)), all after a 60-minute run-in rest time. Boschmann et al. followed a 15-minute lead-in with 120 minutes of RMR testing, (30 minutes of baseline RMR and 90 minutes postdrinking) for both studies (Boschmann et al., 2007; Boschmann et al., 2003). We note that our study had the shortest RMR measurement time (n = 45 minutes) and could have influenced the trends. Nevertheless, in developing our protocol, we found that participants began to fidget after 40–50 minutes under the hood with a 30-minute run-in. Furthermore, we found that many participants began to be uncomfortable and expressed a need to void about 50 minutes post-water-ingestion. The timeline we chose seemed appropriate as data appeared to have peaked well before the conclusion of our RMR tests.

### Limitations

This study had several strengths, such as objective and valid measurements, a large sample size, and a randomized crossover design. Even so, there are weaknesses in this study. First, our findings are limited to adults aged 18–40 years old, who reported being weight stable, who do not excessively consume alcohol or use tobacco or recreational drugs, and who are generally healthy and free of metabolism-altering medications. Our findings do not include pregnant or lactating women. A relationship may be present in other populations. Second, our findings only apply to acute RMR in response to the consumption of 500 ml of water at 3 °C. An intervention that included additional water consumption (as shown in other studies) could be beneficial to outcomes of interest not examined in our study (e.g., weight management). In addition, colder or warmer water could have yielded different results, (as found in another study (Boschmann et al., 2007)). Third, a larger sample size may have been able to detect small differences. We calculated our sample size based on a low-moderate effect found by earlier researchers, but we and others have not been able to detect the same relationship given these effect sizes.

#### Conclusion

In this sample, our findings did not support our hypothesis that consumption of 500 ml of water would have a measurable effect on RMR compared to not consuming water. Indeed, we found no significant difference in RMR in response to consuming water. As such, one positive application of these findings is that water may be a suitable control in RMR studies. Nevertheless, these results should not discourage overall water consumption nor do we suggest that sufficient water consumption is not healthy. In addition, these results do not suggest that consumption of water-rich foods over time is not an effective strategy for weight management

(as shown in other studies). Rather, our study simply replicates other studies that suggest there is not an acute measurable effect of water consumption on RMR. Future studies could attempt to determine if larger volumes of water or different temperatures of water have an effect.

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Variable	Ν	
Sex (F;M)	23;20	NA
Race (Caucasian)	37	NA
Age (years)	43	$27.98 \pm 7.06*$
Height (cm)	43	$171.09\pm9.75$
Weight (kg)	43	$82.58 \pm 15.44$
BMI	43	$28.20\pm4.59$
Resting HR (bpm)	42**	$67.75\pm8.92$

Table 1. Demographic variables

\*Where pertinent, data are expressed as mean  $\pm$  SD. \*\*Resting heart rate data was not available for one participant.

Condition	No Water			Water	F	р
	Ν	Mean	Ν	Mean		
Hunger	43	$40.85\pm22.49$	41	$40.93\pm24.13$	0.01	0.9433
Thirst	43	$58.81\pm22.65$	41	$59.66\pm21.64$	0.01	0.9115
Fullness	43	$32.07\pm19.49$	41	$30.80\pm20.66$	0.22	0.6449
How Much Could Eat	43	$49.26\pm18.13$	41	$49.54 \pm 16.80$	0.04	0.8472
Specific Gravity	42	$1.02\pm0.05$	41	$1.02\pm0.01$	0.75	0.3928
Resting Heart Rate	42	$68.03\pm8.19$	41	$67.46 \pm 19.70$	0.40	0.5317

Table 2. Hunger and thirst, hydration, and resting heart rate by condition

Data are expressed as mean  $\pm$  SD.

Note 1: Hunger, Thirst, Fullness, and How Much Could Eat were measured using Visual Analog Scales (VAS); VAS results are in millimeters ranging from 0 (not at all) to 100 (extremely).

Note 2: Hunger = "How hungry do you feel right now;" Thirst = "How thirsty do you feel right now;" Fullness = "How full do feel right now;" and How much could eat = "How much do you think you can eat right now?"

Note 3: Data unavailable for several outcomes.

			No Control for Linearity		Control for Nonlinearity		Model 1†		Model 2‡	
	No Water	Water	F	р	F	р	F	р	F	р
VO <sub>2</sub> (ml/kg/min)	$3.27\pm0.60$	$3.27\pm0.52$	0.68	0.4129	0.69	0.4107	0.62	0.4362	0.81	0.3745
METS	$0.93\pm0.17$	$0.93 \pm 0.15$	0.68	0.4129	0.69	0.4107	0.62	0.4362	0.81	0.3745
RQ	$0.8095\pm0.05$	$0.8083\pm0.06$	13.73	0.0006	14.20	0.0005	13.21	0.0008	13.78	0.0006
REE (kcal/d)	$1836\pm370$	$1828\pm358$	3.49	0.0688	3.51	0.0682	3.17	0.0825	2.44	0.1256

# Table 3. Metabolic variables by condition

Data are expressed as mean  $\pm$  SD. One MET = 3.5 ml/kg/m<sup>2</sup>

†Model 1: control for nonlinearity and hydration (specific gravity).

‡Model 2: control for nonlinearity, age, sex, and weight.

					Model 1†		Mod	lel 2‡
	No Water	Water	F	р	F	р	F	р
VO <sub>2</sub> (ml/kg/min)	$3.24\pm0.54$	$3.26\pm0.51$	1.40	0.2435	1.37	0.2488	1.38	0.2467
METS	$0.93\pm0.15$	$0.93\pm0.15$	1.40	0.2435	1.37	0.2488	1.38	0.2467
RQ	$0.8095 \pm 0.051$	$0.8111\pm0.06$	10.57	0.0023	9.70	0.0034	10.48	0.0024
REE (kcal/d)	$1822\pm337$	$1827\pm345$	0.18	0.6749	0.17	0.6791	0.36	0.5535

Table 4. Metabolic variables by condition (removal of the first 5 minutes)

Data are expressed as Mean  $\pm$  SD. One MET = 3.5 ml/kg/m<sup>2</sup>

<sup>†</sup>Model 1: control for hydration (specific gravity).

‡Model 2: control for age, sex, and weight.

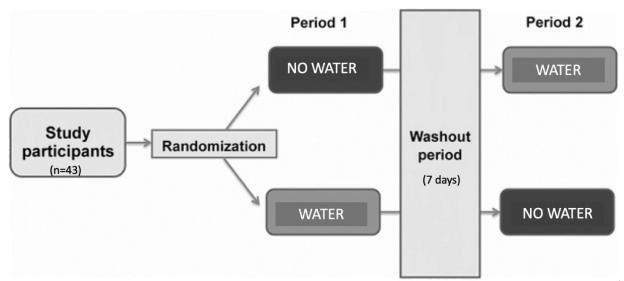


Figure 1. Study Design

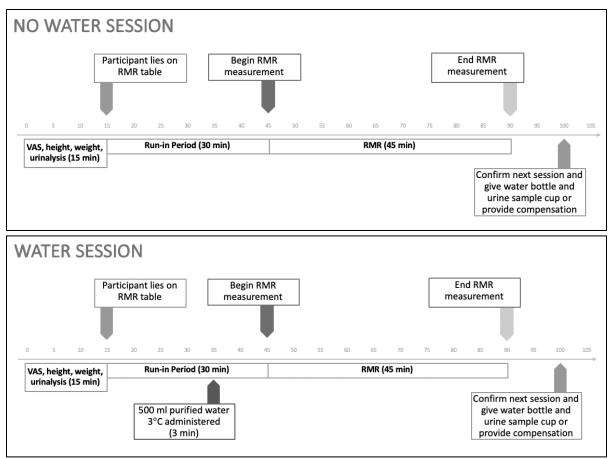


Figure 2. No Water and Water Condition Protocols

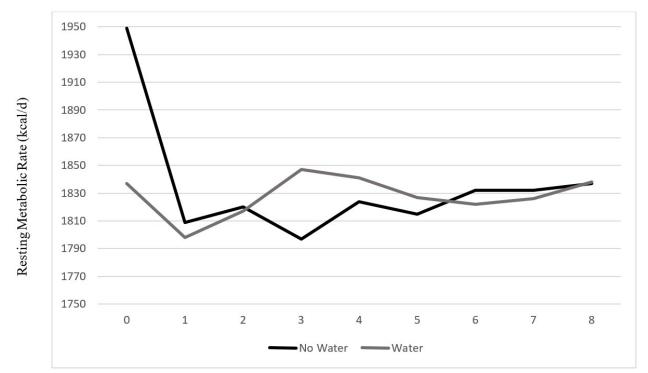


Figure 3. Resting Energy Expenditure Across 45 Minutes

Note: Data are reported in 5-minute intervals: 0 = 1-5 minutes; 1 = 6-10 minutes; 2 = 11-15 minutes; 3 = 16-20 minutes; 4 = 21-25 minutes; 5 = 26-30 minutes; 6 = 31-35; 7 = 35-40; 8 = 41-45 minutes.