Effect of Nighttime Eating on Next Morning Hydration Status and Running Performance in Female Endurance Athletes

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EFFECT OF NIGHTTIME EATING ON NEXT MORNING HYDRATION STATUS AND
RUNNING PERFORMANCE IN FEMALE ENDURANCE ATHLETES

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This thesis is dedicated to increasing the scientific literature base for female endurance athletes, as human performance studies are far more abundant in the male population.
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ABSTRACT

Background: The nutrient content and fluid retentive capacity of chocolate milk (CM) is beneficial for most athletes. However, nighttime consumption of CM has an unknown impact on next morning endurance performance and hydration status. Preliminary evidence suggests that carbohydrate, casein and whey proteins are efficiently metabolized at night; therefore, nighttime consumption of CM may prime athletes for next morning competition. Purpose: To investigate the influence of nighttime consumption of CM on next morning hydration status and running performance in female endurance athletes. Methods: Twelve female runners (age, 30 ± 6 years; running 30.5 ± 11.5 miles/week; VO2max, 52.8 ± 4.2 ml/kg/min) participated in this randomized, crossover, double-blind study. After one familiarization trial, two experimental trials were completed. Participants consumed either skimmed CM or flavor-matched placebo (PLA) two hours after the last meal and 30 minutes before sleep. The next morning, participants arrived to the laboratory following an overnight fast. A visual analogue scale was used to measure subjective ratings of appetite. Urine output (UO), urine specific gravity (USG), and nude body weight was measured. Following a standardized, progressive 20-minute warm up, participants drank 8 ounces of water and then ran a 10-km treadmill time trial (TT). Participants blindly controlled speed. The only known measure was distance completed. Finish time, UO, USG, and nude body weight were measured post-exercise. Repeated measures analysis of variance, Students T-tests (SPSS, Cary, NC) and statistical inferences were used to compare results. Significance was set to p<0.05. Values are reported as means ± SD. Results: Desire to eat was significantly lower the morning after CM consumption compared to PLA (CM, 45.8 ± 23.5 mm vs. PLA, 55.6 ± 22.8 mm; p= 0.04). Although values were not statistically significant, subjective rating of hunger was lower (CM, 41.2 ± 21.0 mm vs. PLA, 48.6 ± 25.2 mm; p=0.31)
and satiety was higher (CM, 44.8 ± 17.1 mm vs. PLA, 35.9 ± 21.8 mm; p=0.12) after CM. No differences were found for the first morning UO (p=0.15) or post-exercise UO (p=0.29). TUO did not vary significantly (p=0.10), however, it was lower for CM than PLA (CM, 718.1 ± PLA, 260.8 vs. 874.8 ± 179.6 ml) and the probabilistic inference of the true, large-sample effect stated that the CM treatment would likely cause the TUO to decrease. Changes in body weight from before and after the TT did not differ (CM, 1.05 ± 0.35 kg, p=0.91). First morning USG did not vary between CM and PLA (CM, 1.015 ± 0.006 vs. PLA, 1.013 ± 0.007; p=0.14) and post-exercise USG was identical for CM and PLA (CM, 1.011 ± 0.006 vs. PLA, 1.001 ± 0.006; p=0.91). TT finish time was 3.24 seconds faster for CM (53.3 ± 7.9 minutes) than PLA (53.4 ± 8.2 minutes), but results were not statistically significant (p=0.95). Heart rate, Rating of Perceived Exertion, and average speed during the TT did not vary between CM and PLA.

**Conclusion:** Nighttime (pre-sleep) consumption of CM reduces next-morning desire to eat and does not impair 10-km TT running performance in female endurance athletes. After consuming CM or PLA, athletes woke up adequately hydrated (USG of ≤ 1.020) and remained hydrated throughout the 10-km TT. The nighttime beverage did not have a detrimental effect, so it may be a useful solution to help female athletes meet nutritional and hydration needs for race morning.
INTRODUCTION

Fueling for optimal performance has become an important component in the training lifestyle of the modern endurance athlete. As training techniques become more advanced, sport-specific nutritional strategies are sought after to gain a performance advantage. Nutrient timing is one approach to maximize training quality, recovery, and ultimately endurance performance. While much research has focused on nutrient intake in close proximity to training (pre-, during-, post-training), evidence is starting to mount which suggests that nighttime eating (specifically, within 30 minutes before sleep) may be beneficial for active individuals. Interestingly, eating before sleep may offer an additional “window of opportunity” for endurance athletes to optimize next morning performance and hydration status, which is when most endurance sports begin. Despite the practicality of eating before sleep for endurance performance, a paucity of data exists at present.

New research suggests the potential for a broad range of health outcomes as a result of nighttime eating. Specifically, the nighttime consumption of a low-calorie (~150-200 kcals), nutrient dense meal composed primarily of a single macronutrient (i.e. protein) suggests enhanced body composition and overnight muscle protein synthesis (MPS) in both clinical and healthy populations. As evidence begins to mount that nighttime eating may be beneficial for active individuals, it is logical to theorize that it may also impact exercise performance.

High-intensity endurance exercise is characterized by elevated muscle glycogen utilization, protein degradation, and hydration needs. It is well documented that endurance performance can be optimized if the athlete begins exercise with high muscle glycogen stores, a positive whole-body protein balance, and is adequately hydrated.
As most competitive endurance events begin early in the morning, there is a limit to the amount of food or beverage an athlete can consume prior without disrupting normal sleep patterns or risking gastrointestinal distress during competition. For this reason, many endurance athletes do not eat much, if anything, before competitions of less than 75-90 minutes in duration. Unfortunately, this could result in an endurance athlete beginning competition in a sub-optimal physiological condition. The incorporation of a pre-sleep meal may provide an added “window of opportunity” for optimizing pre-race glycogen stores, protein balance, and hydration.

Nighttime consumption of a carbohydrate-protein-electrolyte (CP) beverage, specifically chocolate milk (CM), has not been studied and therefore has an unknown impact on next morning endurance performance. However, it is well documented that the relative macronutrient balance and fluid retentive capacity of CM or comparable CP beverage makes for an effective post-endurance exercise recovery aid\(^3,5–7,10–12,29\). There is also evidence to suggest that CM is an effective nutritional strategy before and during endurance exercise\(^8,30\). CM generally provides adequate carbohydrate, protein (casein, whey, and branch chain amino acids), and electrolytes (sodium and potassium) to address system needs before, during, and most notably, after exercise\(^3,5–8,10–12,29,30\). As there is preliminary evidence to suggest that carbohydrate, casein and whey proteins are efficiently metabolized at night\(^13,14,16,17\), CM may be an effective supplement for the nighttime feeding scenario.

The outcomes from this study are particularly relevant to female endurance athletes that are notoriously under-researched. Interestingly, while performance is paramount, nighttime eating may also provide additional benefits for exercise recovery. To date, no studies have examined the effects of whole-foods, like chocolate milk (CM), on next morning performance and hydration status of endurance athletes or women.
Purpose

The purpose of this study was to determine the impact of nighttime eating of CM, as compared to flavor-matched, non-nutritive PLA, on the next morning hydration status and 10-kilometer (km) running performance in female endurance athletes.

Specific Aims and Hypotheses

Specific Aim 1: Evaluate the running performance the morning after nighttime eating of CM vs. PLA.

- Performance was measured by time to complete a 10-km treadmill running time trial (TT).

Hypothesis 1: Consumption of the CM will result in an increased running performance compared to PLA, as indicated by a faster finishing time in the 10-km TT.

Specific Aim 2: Assess the next morning hydration status after nighttime eating of CM vs. PLA.

- Morning hydration status was measured by urine specific gravity (USG) using a handheld clinical refractometer (ATAGO® Pocket PAL-10S) and by total urine output the next morning.

Hypothesis 2: Next morning USG will be lower following consumption of CM vs. PLA, indicating a more optimal pre-exercise hydration status. Also, the total urine output will be lower after CM, indicating greater fluid retention.

Specific Aim 3: Determine differences from consumption of CM vs. PLA on post-exercise hydration status.
• Post exercise hydration status was determined by measuring the USG after
the 10-km TT and by calculating changes in nude body mass from before
and after exercise.

_Hypothesis 3:_ The post-exercise USG will increase to a greater extent with the PLA than
with the CM and the change in nude body mass will be less with the CM.

_Assumptions_

The following assumptions were made in this study:

1. All participants accurately recorded their medical history, dietary and fluid intake, and
   endurance training history, and adhered to the conditions determined in the Informed
   Consent.

2. All participants complied with the instructions for treatment beverage intake as set
   forward in the Informed Consent and as directed by the researchers.

3. All participants accurately monitored their menstrual cycle so determinations of their
   current phase could be made.

4. All participants recorded accurately on their food, beverage, and exercise logs three
days preceding each experimental trial.

_Limitations_

The major limitations of this study included the following:

1. Diet and fluid intake was self-selected and self-reported.

2. Physical activity outside of laboratory protocols was self-controlled.
3. At the time of testing, the menstrual cycle phase was determined based on the approximate day of the menstrual cycle, not by direct hormonal measurement.

4. Full consumption of the treatment beverages at the proper time was based on honor of the participant and was not monitored by a researcher. Participants were asked to bring in the empty container as evidence of consumption.

**Delimitations**

Delimitations of the present study included the following:

1. The study included 12 females between the ages of 18 and 40 years old considered ‘moderately aerobically trained,’ defined as a weekly running mileage $\geq 25$ miles for at least 6 months, and a VO$_{2\text{max}}$ $\geq 45$ ml/kg/min. Additionally, participants must have either consistently used oral contraceptives (greater than 2 months) or have been considered eumenorrheic without oral contraceptive use.

2. Females who were lactose intolerant, had an irregular menstrual cycle, were smokers (current or quit within the last 3 months), those with uncontrolled thyroid conditions, those who took any dietary supplements intended to improve performance, those who took anti-inflammatory drugs regularly or those who had a musculoskeletal injury that could limit performance were excluded from this study.

**Definitions of Terms and Abbreviations**

ATP – Adenosine Triphosphate. The universal energy molecule for muscle contraction that is produced when fuel is broken down. ATP is regenerated primarily through aerobic metabolism within the mitochondria of the cells to provide energy to the muscles for endurance activities$^{31}$. 
**BCAAs** – Branched chain amino acids. The amino acids leucine, isoleucine, and valine make up the BCAAs. These amino acids are important in triggering muscle protein synthesis\(^{32}\).

**BODPOD** – Product used to complete body composition assessments via air displacement plethysmography. Body composition is measured by body density and body fat percentage and is calculated using the Siri or Brozek equations\(^{33}\).

**Casein Protein** – The major protein found in bovine milk (~80% of milk proteins). Casein proteins are digested and absorbed slowly, as compared to its counterpart, whey protein\(^{32}\).

**CM** – Chocolate milk beverage.

**CP** – Carbohydrate-protein beverage.

**EAAs** – Essential Amino Acids. Amino acids (phenylalanine, valine, threonine, methionine, tryptophan, histidine, isoleucine, leucine, and lysine) that must be consumed through the diet\(^{34}\). Essential amino acids are vital for muscle protein synthesis\(^{32}\).

**Eumenorrheic** – A normal menstrual cycle, covering a period of 26-35 days\(^{35}\).

**Follicular Phase** – The first half of the menstrual cycle, which is predominated by follicle stimulating hormone and leuteinising hormone. The follicular phase begins with the onset of menstruation and ends with ovulation\(^{35}\).

**IET**- Incremental Exercise Test. Exercise test that consists of multiple stages that increase in intensity based on the oxygen uptake of the subject\(^{36}\).

**Luteal Phase** – The second half of the menstrual cycle that is characterized by an increase in oestrogen and progesterone. The luteal phase begins after ovulation, which typically occurs 7 days following the first day of menstruation (or halfway through the entire cycle)\(^{35}\). It ends with the onset of a new cycle.
**MPS** – Muscle Protein Synthesis.

**Muscle Protein Balance** – Also known as muscle protein turnover, muscle protein balance is a synchronized, continuous process that occurs at all times in human muscle. A net protein balance of zero is kept when muscle protein synthesis equals that of muscle protein breakdown. Skeletal muscle mass will remain unchanged when protein balance is zero, will decrease when it is negative, and will increase when it is positive\(^{37}\).

**OC** – Oral Contraceptives. Pills that block the normal hormonal feedback mechanisms of the menstrual cycle and inhibit ovulation. Oral contraceptives can be used to control or stabilize the influence of the menstrual cycle on other physiological systems\(^{35}\).

**PLA** – Non-nutritive, flavor-matched placebo.

**RER** – Respiratory Exchange Ratio. The rate of carbon dioxide produced compared to the amount of oxygen consumed. RER gives an indication to the main type of fuel source (foodstuff) being utilized at rest and during exercise\(^{38}\).

**RMR** – Resting Metabolic Rate. The amount of energy needed to sustain life at rest, measured as kilocalories per day\(^{39}\).

**RPE** – Rating of Perceived Exertion. A subjective rating system for exercise intensity based on general fatigue\(^{39}\).

**TT** – Time Trial. An exercise test in which subjects complete a preset amount of work as fast as possible\(^{40}\).

**TTE** – Time-to-Exhaustion. An exercise test performed at a constant speed until the subject can no longer maintain the required work rate\(^{41}\).
**Urine Osmolality** – A measurement used to assess hydration status. A urine osmolality of $\leq 400$ mOsmol/kg is considered normal hydration status$^{42}$.

**USG** – Urine Specific Gravity. An index of hydration status, measured using a refractometer. A first morning void of $\leq 1.020$ is considered normal hydration status$^{42}$.

**VO_{2\text{max}}** – Maximal Oxygen Uptake. The highest rate at which the body can take up and consume oxygen during intense exercise. VO$_{2\text{max}}$ is typically viewed as one of the most important predictors of endurance performance$^{31}$.

**Whey Protein** – The liquid protein portion of bovine milk. Whey protein is digested and absorbed rapidly, as compared to casein protein$^{32}$.
REVIEW OF LITERATURE

Nighttime Eating

Nighttime eating is a relatively new phenomenon in the arena of sports nutrition. Most original research in this area stems from nighttime shift workers\textsuperscript{43} or individuals with nighttime-eating disorders\textsuperscript{44,45}, with primarily negative outcomes for cardiovascular disease factors and body composition. However, these outcomes may have been dependent on factors other than nighttime eating itself. For example, consumption of nighttime meals has also been associated with a low daily frequency of meals\textsuperscript{43}. Low meal frequency is associated with low resting metabolic rate (RMR) and nighttime consumption of high calorie (~700 kcal) and non-nutrient dense meals\textsuperscript{44}. It may then be the case that overall diet and the quality of a nighttime meal may dictate potential outcomes more greatly than the presence of a nighttime meal alone.

This notion is evidenced by more recent research that suggests physiological benefits from nighttime feeding in populations that are not shift-workers\textsuperscript{13–18}. In these controlled studies, where treatment meals were small (~150-200 kcals), nutrient dense, and contained only one single macronutrient, outcomes were positive. Interestingly, favorable outcomes from a nighttime feeding have been established within an obese population. These results include increases in next morning satiety levels and insulin resistance\textsuperscript{18}, as well as decreases in systolic blood pressure and arterial stiffness\textsuperscript{17}. Madzima et al. (2013) examined the effect of acute nighttime supplementation of various protein and carbohydrate treatment beverages on next morning measures of RMR in healthy young physically active men\textsuperscript{13}. It was found that regardless of substrate (casein protein, whey protein, or carbohydrate), there was an increase in RMR compared to placebo. Continually, nighttime eating of casein protein was demonstrated to increase the rate of muscle protein synthesis (MPS) and net protein balance in elderly men\textsuperscript{16}. In
a follow up study, similar results were reported in a young, resistance trained population\textsuperscript{14}. Taken together, these data demonstrate that not only can protein be digested and metabolized normally during sleep, but also that it directly impacts MPS and recovery from exercise. Thus, nighttime eating has shown the potential for a broad range of health benefits including body composition and metabolic health in several populations. It is also logical to theorize that nighttime eating may improve exercise performance.

The spectrum of literature regarding nighttime eating in athletes is extremely limited. As mentioned previously, results from Madzima et al. (2013) and Res et al. (2012) are promising, as both studies used young and recreationally active populations\textsuperscript{13,14}. Acute increases in RMR were reported by Madzima et al. (2013); if chronic supplementation continued this trend, positive net changes to body composition might suggest an indirect performance gain, specifically in aerobic and weight-bearing sports\textsuperscript{46,47}. Nighttime feeding may further indirectly benefit performance by increasing absorption of carbohydrate. A study concerning late night supper (2300 hours) versus traditional timing (1800 hours) in Japanese students reported increased efficiency in next morning carbohydrate digestion and absorption\textsuperscript{48}. If apparent in athletic populations, this is a potential added benefit to the endurance athlete, as enhanced carbohydrate absorption from a small nighttime meal could maximize pre-race glycogen stores and enhance carbohydrate utilization during exercise. Beginning exercise with a maximal amount of muscle glycogen has been shown to improve performance\textsuperscript{19,22}. Similarly, the fact that protein can be absorbed effectively at night and promotes MPS\textsuperscript{14} has relevant application for recovery from exercise both acutely\textsuperscript{49} and habitually\textsuperscript{50}.

Most competitive endurance events begin early in the morning, which limits the amount of food or beverage an athlete can consume without causing gastrointestinal distress during
competition. For this reason, many endurance athletes do not eat much, if anything at all, before competition. This potential nighttime “window of opportunity” may provide an optimal nutritional strategy for the athlete to begin exercise with high glycogen stores, in positive protein balance, and fully hydrated.

Factors Affecting Endurance Performance

Endurance exercise includes activities such as long distance running, cycling, swimming, distance rowing, and cross-country skiing. In order to understand the potential benefits of nighttime eating on endurance performance, it is necessary to first understand what limits endurance performance. Multiple factors play a role in either enhancing or compromising endurance performance. Some of the main factors that can affect performance include available fuel, positive muscle protein balance, adequate hydration status and, particularly to female athletes, the menstrual cycle.

Fuel Availability

Successful endurance exercise relies on adequate amounts of energy in the form of adenosine triphosphate (ATP) to support metabolism and muscle contraction. The main fuel sources that provide this energy include dietary fats and carbohydrates\textsuperscript{31,51}. Intensity and duration of the endurance activity will determine whether carbohydrate or fat is the main fuel source. Intensity of an endurance activity can be measured by the percentage of one’s maximal oxygen uptake (VO\textsubscript{2max}). VO\textsubscript{2max} is defined as the highest rate that the body can take up and utilize during intense exercise\textsuperscript{31}. At lower exercise intensities (<60% of VO\textsubscript{2max}), muscle metabolism is supported by a combination of fuel from fat and carbohydrate. However, as the intensity increases, the body begins to rely heavily on carbohydrate as the main source of fuel\textsuperscript{20,22,31,51,52}. 

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Most competitive endurance activities are completed at an intensity higher than 70% VO$_2$max, indicating that the athlete must rely mostly on their blood glucose and muscle glycogen concentrations in order to successfully compete$^{31}$. Adequate carbohydrate must be available in the diet prior to the start of exercise to maintain blood glucose and maximize glycogen stores in the muscle and liver$^{51}$. Several studies have reported that endurance capacity is reduced when blood glucose is low and muscle glycogen stores are depleted$^{2,20-22,53}$. Bergstrom et al. (1967) demonstrated this phenomenon by manipulating the diets of endurance-trained cyclists in order to vary the levels of their muscle glycogen stores. The cyclists with the higher glycogen content were able to perform to a higher level than their counterparts with reduced muscle glycogen when completing a cycle ergometer test at 75% of VO$_2$max$^{19}$. Walker et al. (2000) tested time to exhaustion of endurance-trained women with high and low glycogen stores working at 80% of their VO$_2$max. Glycogen utilization was greater and the time to exhaustion (TTE) extended for the women with higher glycogen stores$^{22}$. Lastly, cyclists that were fed carbohydrate during prolonged endurance exercise at 70% VO$_2$max were able to extend TTE and increase carbohydrate utilization compared to subjects who were not fed carbohydrate$^{2}$. The culmination of these studies indicates that endurance performance completed at high-intensity relies heavily on the amount of muscle glycogen and blood glucose concentrations available to muscles during exercise.

**Muscle Protein Balance**

In order to support muscle tissue growth and repair, it is imperative for muscles to obtain positive muscle protein balance. Protein balance is determined by the rate of MPS compared to the rate of muscle protein breakdown. Dietary protein provides amino acids that are necessary for the building and repair of muscle tissue$^{23,50,51}$. 
Most importantly, the availability of essential amino acids (EAAs) from dietary protein is a key factor in achieving positive muscle protein balance\textsuperscript{49,54–56}. It has been demonstrated that the ingestion of EAAs will cause a rapid rise in blood amino acid concentrations, which increases the rate of delivery to the skeletal muscles, and therefore, will increase the rate of MPS. The stimulation of MPS seems to depend mostly on the amount of amino acids available in the blood, not on the amount of amino acids available in the intramuscular space\textsuperscript{37,54,55}. Bohé et al. (2003) showed that there was little increase in MPS as intramuscular EAA concentrations rose; however, a substantial positive curvilinear rise in MPS occurred as blood EAAs increased. Therefore, indicating the importance of consumption of all EAAs through the diet in order to signal the pathway of MPS\textsuperscript{54}.

![Figure 1. Schematic of muscle protein synthesis and breakdown\textsuperscript{37}](image)

Figure 1 depicts the flow of amino acids through the blood and intracellular amino acid pool\textsuperscript{37}. Without the contribution of dietary amino acids, there would be no supply of amino acids coming from the blood, leading to significantly lower amounts available for MPS. Dashed
arrows represent the direction of amino acids broken down from food, entering the bloodstream, taken up into the muscle, and then ultimately contributing to the muscle proteins\textsuperscript{37}.

Moreover, the type of protein that maximally stimulates MPS has been highly researched. While evidence is not clear-cut, it appears that both whey and casein proteins have the potential to stimulate MPS. Indeed, Tipton et al. (2004) reported that the consumption of these milk proteins following resistance exercise increased muscle protein balance. Both whey and casein contain all EAAAs necessary for MPS\textsuperscript{49}.

All in all, it is important for endurance athletes to maintain appropriate dietary protein intake to minimize the risk of a negative protein balance, as decreases in endurance performance have been noted in athletes in negative muscle protein balance\textsuperscript{23}.

\textit{Hydration Status}

Adequate fluid balance prior to exercise is necessary in order to prevent performance decrements\textsuperscript{51}. A water deficit in the body of just 2-3\% can induce alterations in cardiovascular function, thermoregulatory function, central nervous system activity, and metabolic functions\textsuperscript{25,26,28,31,51}. These disturbances to the body’s normal functions can inhibit aerobic capacity and decrease performance. Increases in heart rate (HR), decreases in stroke volume, higher core temperatures, and higher ratings of perceived exertion (RPE) were all observed in hypohydrated athletes\textsuperscript{26–28}. This increased cardiovascular strain may lead to decreased motivation or physical ability to exert a maximum effort, resulting in less than optimal performance.

Davis et al (2014) demonstrated the importance of proper pre-exercise hydration in a 10-km running TT performed by trained men and women. Running performance of two different
groups was compared, between which the pre-exercise urine specific gravity (USG) varied significantly (P<0.001). One group began the time trial with a USG of 1.014 (adequate), whereas the other began with a value of 1.026 (dehydrated)\(^5\). (According to the ACSM Position Statement, first-morning USG ≤1.020 is considered to be adequately hydrated\(^4\)). The group that began exercise is an adequately hydrated state had a 3% increase in overall performance as compared to the dehydrated group (Adequate: 4.60±0.60 min/km; Dehydrated 4.73±0.66 min/km; P=0.001). This performance increase was due to a significantly faster pace in the second half of the run (Lap 2 Pace: Adequate 4.69±0.69 min/km vs. Dehydrated 4.93±0.80 min/km; P=0.002). Additionally, the hydrated group had a significantly lower RPE than did the dehydrated group throughout the run (7.5±1.3 vs. 8.4±0.9; P=0.02)\(^5\). Similar outcomes were seen in a 12-km running TT, in which a pre-run hydrated state resulted in significantly faster finishing times as compared to a pre-run dehydrated state (Hydrated: 53.15±0.6.05 minutes; Dehydrated: 59.44±5.44 minutes; P<0.01)\(^5\).

Furthermore, the rate of muscle glycogen depletion during exercise may be accelerated in a dehydrated state as compared to a hydrated state\(^26,28\). Hargreaves et al (1996) found that athletes who remained hydrated throughout exercise used 16% less muscle glycogen than those who were dehydrated\(^28\). The faster the depletion of muscle glycogen, the sooner fatigue will occur, ultimately, impairing performance. As such, athletes should strive to begin exercise in a hydrated state in order to maximize their endurance performance.

*The Menstrual Cycle*

When conducting studies in women, it is important to understand the physiology of the menstrual cycle and whether or not the hormonal fluctuations impact performance. On average, the cycle lasts between 26-35 days, and is divided into the follicular and luteal phase. The
The follicular phase begins with the onset of menses (day 1 of the cycle) and ends about 14-15 days later, after ovulation occurs. The luteal phase begins after ovulation occurs (about day 15 of the menstrual cycle)35.

The main acting hormones during the follicular phase are follicle stimulating hormone (FSH) and luteinizing hormone (LH), which gradually increase throughout the first half of the cycle and surge upward with ovulation. Prior to ovulation, gonadotropin releasing factor stimulates the growth of a follicle in the ovary. The ovary responds to the growing follicle by releasing estrogen, which then causes the drastic increase in LH (accompanied by a less drastic increase in FSH) 24 hours prior to ovulation. The luteal phase begins the day after ovulation, and is characterized by elevated levels of estradiol and progesterone. The cells that remain in the ovary following ovulation produce these hormones and prevent further production of FSH and LH. If fertilization does not occur throughout the 14 days of the luteal phase, the cycle will repeat itself, marked by the onset of menses35.

Figure 2. Timing of the menstrual cycle.
It has been proposed that the hormonal fluctuations throughout the menstrual cycle alter ventilation, metabolism, thermoregulation, and motivation, all of which could potentially alter endurance performance\textsuperscript{35,59}. However, despite these alterations, culminations of studies have indicated that neither maximal\textsuperscript{59–61} nor submaximal\textsuperscript{60–63} endurance exercise performance is affected by the menstrual cycle phase. Oosthuysse et al. (2005) demonstrated this by having a group of trained female cyclists complete a 30-km cycling TT during the early follicular phase, late follicular phase, and mid-luteal phase of the menstrual cycle. There was no significant difference in the intensity at which the women cycled (71-80% VO\textsubscript{2max}) or in their TT performance between phases\textsuperscript{63}. This same occurrence was observed in several TTE protocols. The phase of the menstrual cycle did not affect cycling\textsuperscript{60,62} or rowing\textsuperscript{61} TTE at intensities between 70-85% VO\textsubscript{2max}. In addition to performance, the menstrual cycle did not alter the HR responses to exercise or the RPE\textsuperscript{60,61,63}.

Likewise, the typical increase in progesterone during the luteal phase is associated with an increase in core body temperature\textsuperscript{64}. It becomes a concern that this increased core temperature may increase cardiovascular strain, induce dehydration, and in return, decrease performance. Garcia et al. (2006) found that, despite the increased pre-exercise core temperature in the luteal phase, the same temperature was maintained throughout submaximal cycling exercise in both the follicular and luteal phase. The body was able to adapt by increasing the sweat rate during the luteal phase, leading to no difference in HR responses or RPE between menstrual phases. Additionally, the pre-exercise USG, pre-exercise urine volume, and whole body fluid balance was the same in both the luteal and follicular phases, indicating that the phases did not affect hydration status\textsuperscript{64}. Similar results were presented in NCAA Division 1 female athletes, showing no difference in pre-exercise USG during the follicular or luteal phases\textsuperscript{65}. Therefore, it is
apparent that menstrual cycle phase does not appear to impact endurance exercise performance or hydration status of female athletes.

*Oral Contraceptives*

Due to the equivocal results related to the menstrual cycle, oral contraceptives (OC) are often used to promote a more stable hormonal environment for the female athlete. OC use helps to regulate the length of the menstrual cycle and maintain normal hormonal fluctuations throughout its entire course. Multiple studies have investigated the effect of OC use in general, as well as within specific phases of the OC cycle, on endurance performance. When evaluated, no difference in cycling performance or anaerobic threshold was detected between women using OC and eumenorrheic women. Females taking OC experienced lower peak VO₂ values at the anaerobic threshold, however, the intensity at which anaerobic threshold occurred did not vary significantly between the two groups. Also, the most important outcome to take note of is that no difference in TTE was recorded between OC users and non-users. Similar results have been reported in female rowers, showing no change in maximal or submaximal exercise performance measures for OC users. Lastly, when active and non-active phases of the OC pill cycles were compared, no differences in endurance performance or substrate use were documented. Thus, female endurance athletes taking OC do not need to worry about altered performance outcomes. Although OC research is at times conflicting, the above results show reasonable support for OC use to minimize performance differences between stages of the menstrual cycle in female endurance athletes.

*Milk as an Ergogenic Aid*

Given that the existing research has shown benefits of whey protein, casein protein, and carbohydrate when small portions are consumed before bed, it is logical to pursue milk
as a viable whole-food choice to ingest before bed. Bovine-based milk products provide excellent sources of proteins (including all essential amino acids), natural carbohydrate (in the form of lactose), vitamins, and minerals\textsuperscript{32,70}. A 240 mL serving of skimmed CM, which has become an extremely popular sports nutrition beverage, provides approximately 120 kilocalories, 20g of carbohydrate, 8g of protein, 0g of fat, 180mg of sodium, and 420mg of potassium\textsuperscript{71}. This nutrient profile is comparable to that of several commercially available sports drinks\textsuperscript{70}.

Consumption of milk-based beverages has long been a proposed recovery strategy for endurance exercise\textsuperscript{3,5–7,11,12,26,29}. The relative macronutrient balance and fluid retentive capacity, combined with the fact that it is a whole-food complex, gives milk and milk-based beverages equal\textsuperscript{7} or arguably enhanced\textsuperscript{3,5,6,29} status as a post-exercise recovery aid compared to the traditional commercial performance beverage.

The effects of milk, or a comparable carbohydrate-protein supplementation, on endurance capacity has been investigated. Karp et al. (2006) compared the efficacy of low-fat CM to carbohydrate-only and fluid replacement (containing electrolytes) beverages as a recovery aid following an initial trial of glycogen depleting exercise on a cycle ergometer. Efficacy was based on subsequent performance in a cycling TTE test. TTE was enhanced by 49% and 54% for the CM and fluid replacement as compared to the carbohydrate-only beverage, with no statistically significant differences between CM and fluid-replacement. However, milk and carbohydrate treatments were matched for carbohydrate content only, and were not isocaloric or isonitrogenous. It is unclear whether the results were due to the specific macronutrient components or simply the additional calories of the CM beverage\textsuperscript{12}. Thomas et al. (2009) completed a very similar study, yet this time using treatments matched for caloric content. TTE improved to a greater extent with the CM as compared to both the fluid-replacement and
carbohydrate-only (CM 43% longer than fluid replacement and 51% longer than carbohydrate-only)\textsuperscript{3}. These results are mirrored in more recent literature\textsuperscript{5,6}.

However, not all research supports an enhanced exercise capacity with a similar carbohydrate-protein beverage. Betts et al. (2007) demonstrated that a comparable carbohydrate-protein combo did not elicit increased running TTE when compared to a carbohydrate-only treatment. This is potentially due to the well-known ergogenic capabilities of carbohydrate alone\textsuperscript{7,2}. Nonetheless, it is worth noting that while performance outcomes were not different between treatments, the carbohydrate-protein treatment was at least comparable to the traditional beverage and did not lead to any detriment in performance\textsuperscript{7,2}. This, in combination with other potential benefits of milk as an exercise nutritional strategy, makes milk an attractive exercise supplement.

Newer research has sought to examine the effects of milk or a similar carbohydrate-protein beverage fed prior and during a bout of endurance performance. Alghannam (2011) studied the effects of a carbohydrate-protein beverage compared to an isocaloric carbohydrate-only supplemented before and in between intermittent bouts of soccer specific, glycogen depleting exercise. It was concluded that the carbohydrate-protein treatment increased run time to fatigue and total distance covered compared to carbohydrate alone\textsuperscript{8}. Similar results were demonstrated in a prolonged and variable intensity cycling trial with supplementation of either a carbohydrate-protein or carbohydrate-only beverage just prior and throughout testing. There was a 36% improvement in cycling TTE; however, treatments were matched only for carbohydrate content, not calories\textsuperscript{30}. Conversely, Lee et al. (2008) saw no differences in cycle TTE between a low-fat milk and a carbohydrate beverage given prior and during testing\textsuperscript{7}. Again, though no
apparent benefits were reported using the milk treatment, performance was not hindered, and compares to that of the carbohydrate beverage.

In the nighttime feeding scenario, milk-based beverage consumption would not be directly post-exercise. It is assumed that adequate recovery needs would be met following previous bouts of training, making the nighttime milk beverage an additional, isolated dose. Though rates of glycogen and protein synthesis would not be accelerated in this case, as in the instance of immediate post-exercise consumption\textsuperscript{73}, synthesis is still favorable\textsuperscript{54–56,74}. Most importantly, research has shown that, when fed prior to sleep, this normal rate of synthesis, at least for protein, is maintained (and improved compared to non-caloric placebo) during the nighttime window\textsuperscript{14}. Additionally, there is evidence that a mixed macronutrient nighttime meal may even effect next day absorption of carbohydrates, and in the case of the athlete, maximize utilization of carbohydrate typically fed before and/or during exercise\textsuperscript{48}. Lastly, beverages of similar nature to milk have been shown to be effective prior and during exercise\textsuperscript{8,30}, and lend evidence that a milk-based substrate is beneficial to sport performance in areas other than post-exercise recovery.

It is speculated that an additional dose of a low-calorie (~150-200 kcals), nutrient dense, and primarily carbohydrate-protein meal such as milk might be of particular benefit to an endurance athlete the morning after nighttime supplementation. An explanation of the proposed mechanisms by which milk consumption may elicit favorable performance effects will be discussed below.

\textit{Carbohydrate Content}

As mentioned previously, endurance performance at intensities greater than 70\% \textit{VO}$_{2\text{max}}$ is critically dependent on carbohydrate availability via muscle glycogen stores and blood
glucose\textsuperscript{2,19–22}. Therefore, it is logical that carbohydrates are often a main source of energy for competitive endurance athletes.

Milk contains amounts of carbohydrate comparable to most other commercially available performance beverages\textsuperscript{70}. However, milk carbohydrate is primarily in the form of lactose, while other recovery beverages contain primarily glucose or maltodextrin\textsuperscript{70}. It has been speculated that the different carbohydrate make-up between milk and other recovery beverages could be the reasoning for enhanced performance with a milk beverage\textsuperscript{3}. Though, some research suggests the opposite, claiming that the different carbohydrate composition between milk and the other treatments could be the cause for decreased performance outcomes within the milk condition\textsuperscript{7}. When lactose is digested, it is quickly hydrolyzed to its constituent monosaccharides, glucose and galactose, via the enzyme lactase\textsuperscript{75}. It is generally accepted that glucose is absorbed at a rate of 1.0-1.1 grams/min, while galactose is slower, approximately 0.41 grams/min\textsuperscript{75}. Slower absorption of exogenous carbohydrate, as in the case of lactose, would not be optimal during exercise or possibly in the case of time sensitive post-exercise recovery. However, in the case of nighttime feeding, such time sensitivity would not be apparent. An obvious limitation of the carbohydrate composition of milk is in the case of the lactose-intolerant portion of the athletic population.

\textit{Protein Content}

As discussed, endurance exercise is catabolic to muscle proteins, and a negative protein balance is associated with decreased performance\textsuperscript{23}; it is therefore imperative that athletes offset breakdown via increased dietary consumption\textsuperscript{50}. Milk has a high biological value, meaning that it contains several EAAs, provided by the whole proteins casein and whey\textsuperscript{32}. The ratio in milk of casein and whey proteins is 3:1; this particular make-up may require longer digestion and
absorption, sustaining increased levels of blood amino acids for a longer period of time\textsuperscript{32}. Increased availability is advantageous to increased synthesis\textsuperscript{32,54}.

In the athletic spectrum, the addition of protein to a recovery treatment has, more often than not, resulted in positive protein balance\textsuperscript{4-6,76}. The fact that the digestion and absorption of protein remains intact during sleep potentiates a possible ‘untapped’ opportunity for additional MPS, which is of particular advantage to the athlete\textsuperscript{14,16}. Likewise, it has been suggested that the addition of protein to a carbohydrate recovery beverage may augment glycogen synthesis via greater blood insulin levels\textsuperscript{9}. Given the nighttime feeding scenario, the presence of protein and carbohydrate in milk could substantiate gains in overnight glycogen synthesis.

Aside from enhanced protein synthesis, protein in milk may attenuate muscle protein damage resultant from exercise\textsuperscript{77}; however, not all studies support this finding\textsuperscript{6}. Whey protein in particular contains all BCAAs\textsuperscript{32,70}, and besides primary use as a substrate in MPS, similar to EAAs, BCAAs also suppress protein catabolism\textsuperscript{32}. Luden et al. (2007) studied the effects of a carbohydrate-protein beverage on plasma creatine kinase (CK) levels, an indirect measure of muscle protein damage, in college runners. It was found that those who supplemented with carbohydrate-protein accrued less plasma CK, versus the carbohydrate-only intervention\textsuperscript{77}. However, Ferguson-Stegall et al. (2011) reported no such differences between a milk-based treatment and carbohydrate treatment\textsuperscript{6}. If such mechanism transferred to the nighttime feeding window, decreased protein degradation might not directly impact the current bout of exercise; however, as the athlete is interested in chronic recovery enhancement, it could be beneficial for a subsequent bout of training.
Hydration Capacity

Research suggests that milk may have merit as a fluid replacement beverage. Hydration status is of extreme importance to the endurance athlete and directly influences performance. Fluid-electrolyte content, specifically of sodium and potassium, is directly related to restoration of body water balance. Per 250 mL serving, milk contains similar concentrations of electrolytes as a common fluid replacement beverage: about 133 mg of sodium and 431 mg potassium. Shirreffs et al. (2007) compared the supplementation of milk, water, and a commercially available fluid-replacement beverage on rehydration efficacy during and post cycle exercise. Milk treatment resulted in greater fluid retention than all other conditions. Similar results were found when solutions containing milk proteins and carbohydrate vs. carbohydrate alone were ingested following dehydrating exercise. Whole body net fluid balance was significantly lower for the carbohydrate solution (P<0.01). Also, urine volume after drink ingestion was reduced for the carbohydrate-protein compared to carbohydrate (P<0.01), indicating greater fluid retention from the milk proteins. Most recently, James et al. (2013) displayed almost identical results; showing significantly enhanced whole-body net fluid balance (P<0.01), lower urine output (P<0.01), as well as greater urine osmolality (P<0.05) following a milk proteins plus carbohydrate rehydration beverage versus carbohydrate alone. In this respect, milk may be considered an effective method to replace fluids, and potentially substantiate both pre- and post-exercise hydration for the athlete.

Nonetheless, very different from the traditional fluid replacement beverage is the energy density of milk, primarily due to its protein and fat content. An increased energy density is a proposed advantage of milk as it slows the rate of gastric emptying and therefore promotes fluid retention. A study by Seifert et al. (2006) reiterates this notion, as the addition of protein to
a sports drink showed increased fluid retention in subjects. Retention from the protein-added treatment was approximately 88% versus the carbohydrate-only treatment (~74.9%) and water (~53.2%)\textsuperscript{70}. This lends further evidence to the use of milk as a rehydration strategy. In the nighttime feeding scenario, increased pre-exercise hydration from nighttime feeding would be of particular benefit to the endurance athlete, as early morning training and competition often limit time for adequate hydration.

**Conclusions**

A study investigating nighttime (pre-sleep) eating of milk proteins and carbohydrate on morning endurance performance in female athletes has not been reported or published to date. A serving of fat-free CM may be a useful substrate to provide an additional, low-calorie and nutrient-dense meal to the athlete just prior to sleep.

It is well demonstrated that endurance performance is highly influenced by the carbohydrate availability, muscle protein balance, and hydration status of the athlete. Also, nighttime eating seems to improve protein synthesis and carbohydrate absorption in healthy populations. Therefore, a logical inference can be made that nighttime consumption of CM may increase glycogen and protein synthesis throughout the night, it may improve next morning hydration status, and ultimately, it may improve next morning running performance.

The potential to promote this additional glycogen and protein synthesis, as well as an enhanced hydration status, could be of benefit in a next morning bout of endurance exercise, in a fasted state. This may also be an effective strategy to eliminate the risk for gastrointestinal distress that accompanies a pre-exercise meal. For these reasons, an examination of the effects of nighttime eating and performance is warranted.
METHODOLOGY

Participants

Twelve female endurance athletes from the Tallahassee area were recruited to participate in this study. Major methods of recruitment included posting flyers around town and online, announcements at local track club meetings, and announcements at local races. Inclusion was determined based on maximal oxygen consumption ($\text{VO}_{2\text{max}}$), medical history questionnaire, and specific endurance training criteria. Qualified participants were provided written and oral information describing experimental procedures, as well as risks and benefits to participation prior to giving informed consent. Approval by the Florida State University Institutional Review Board was received prior to all testing.

Inclusion Criteria

All participants were female and between the ages of 18 and 40 years. Participants were either consistently using oral contraceptives (greater than 2 months) or were considered eumenorrheic without oral contraceptive use. Additionally, participants were ‘moderately aerobically trained,’ defined as a weekly running mileage $\geq 25$ miles for at least 6 months, with a $\text{VO}_{2\text{max}} \geq 45$ ml/kg/min $^{62,63}$.

Exclusion Criteria

Females who were lactose intolerant, had an abnormal menstrual cycle, smokers (current or quit within the last 3 months), those with uncontrolled thyroid conditions, those taking any dietary supplements intended to improve performance, those who took anti-inflammatory drugs regularly or those with a musculoskeletal injury that could limit performance were excluded from the study.
Research Design

This study had a randomized, double-blind crossover design. Included in the protocol was a $\text{VO}_{2\text{max}}$ test and three performance trials: one familiarization trial, and two experimental trials. The familiarization served as a practice test to minimize any training effects between experimental trials. The experimental trials were completed within a 2-week period determined by the luteal phase of the menstrual cycle (estimated by testing on approximately days 15-28 of the menstrual cycle, with day 1 being the first day of menstruation)\(^8\). A minimum of 48-72 hours and a maximum of 7 days separated the testing days.

Prior to the first experimental trial, participants were required to complete a detailed 3-day dietary food and exercise record. Participants were instructed to replicate this record exactly prior to the subsequent trial. Food Processor Version 10.13.1 dietary analysis software was used for analysis (McGraw-Hill; New York, NY). Participants were also instructed to abstain from use of non-steroidal anti-inflammatory drugs, caffeine, and/or participation in vigorous activity for at least 24 hours prior to each experimental trial.

Qualification Visit

Participants arrived to the laboratory in the morning, fasted (overnight), and completed the informed consent paperwork, health history questionnaire and specific endurance-training questionnaire. Initial height and weight measurements were completed using a Physician’s Scale with attached stadiometer (Seca, Mexico). Participants were measured barefoot, wearing only a sports bra and athletic shorts. Immediately after height and weight, body composition was measured using air displacement plethysmography (BOD POD; COSMED).
After the initial measures, the VO\textsubscript{2max} test was completed. VO\textsubscript{2max} was determined using a graded treadmill (Woodway PPS Med; Waukesha, WI) exercise protocol and a metabolic cart system (Parvo Medics TrueMax 2400 Metabolic Measurement System; Consentius Technologies; Sandy, UT) to ensure qualification for the study. Gas exchange and ventilatory parameters were measured with the metabolic cart system. The metabolic system was calibrated prior to VO\textsubscript{2max} testing and each of the following trials to the manufacturer’s recommendations. Metabolic systems were flow-calibrated with a 3L calibration syringe (no.5530; Hans Rudolph, Inc.; Kansas City, MO) and gas calibrated with gas mixture of known concentrations of oxygen (O\textsubscript{2}) and carbon dioxide (CO\textsubscript{2}) (16% O\textsubscript{2}; 4% CO\textsubscript{2}; Scott Medical Products; Plumsteadville, PA). Temperature, humidity, and barometric pressure were measured using an indoor climate monitor (Perception II TM; Davis Instruments; Hayward, CA).

Participants were fitted with a nose clip and headpiece, including a mouthpiece with breathing tube attachment to collect expired air and deliver to cart. Participants were fitted with a Polar\textsuperscript{TM} heart rate monitor. The testing protocol allowed participants to select their own pace that was ‘comfortable, but challenging’ for the duration of the test. Each participant began the test by walking for 2 minutes. Then, the participants were given 3 minutes to increase their speed to the personally selected pace for the test. Once the 5 minute warm-up was completed, speed was held constant, while grade was increased at a rate of 2% every 2 minutes \textsuperscript{81}. Heart rate (HR) and rating of perceived exertion (RPE), measured on a 6-20 Borg scale, were recorded during the last 15 seconds of each stage. The test was terminated when the participant could no longer keep pace\textsuperscript{81}. Maximal exercise intensity was defined by the velocity and grade of the last completed stage.
Treatments

Over the span of the study, all participants received the following treatments: 12 oz (355mL) of fat-free chocolate milk (CM) (180 kcal; 12 g PRO; 30 g CHO; 0g FAT) (TruMoo, El Paso, TX) and 12 oz (355mL) of a flavor-matched, non-nutritive placebo (PLA) made from maltodextrin and gums, providing zero calories (Dymatize Nutrition, Dallas, TX). Beverages were prepared by a researcher not involved with this study and provided in identical opaque containers in order to further blind participants to the specific treatment. Empty containers were collected in an effort to verify compliance.

Familiarization and Experimental Trials

Participants did not receive treatment beverages or undergo urine and saliva collection during the familiarization trial. However, all other specifications in this section were exactly the same for the familiarization trial as for the experimental trials.

The day prior to each of the two experimental trials, participants came into the laboratory to obtain the designated treatment beverage. At this time, participants also provided a small saliva sample in order to test the progesterone hormone levels. This sample allowed the researchers to use saliva in order to verify whether the participants were in fact in the luteal phase of the menstrual cycle at the time of testing. Currently to date, saliva samples remain stored in a -20°C freezer until analysis can be completed.

Along with treatment beverages, participants received a urine collection jug to use on the morning of testing. Participants were instructed to use the collection jug for all urine output following consumption of the beverage. They were instructed to completely empty their bladder into the collection jug the morning of testing in order to measure total urine output following
beverage consumption. If the participant needed to urinate additionally after arrival to the laboratory but prior to exercise, urine was collected and added to the pre-exercise total output. After total output was recorded, 0.5 ml of the collected urine was used to measure urine specific gravity (USG) using a digital hand-held refractometer (ATAGO® Pocket PAL-10S). To begin this process, 0.5 ml of water was pipetted onto the refractometer to zero out the device. The researcher wiped the refractometer dry with a delicate task wipe (Kimwipes, KIMTECH Science Brands), and then pipetted 0.5 ml of urine on the device. Once urine is in place, the “start” button was pushed and the refractometer gave the USG reading in approximately 10 seconds. The device was wipe dry and cleaned with a water rinse.

Participants were instructed to drink the beverage at least 2 hours after their last evening meal and within 30 minutes prior to sleep. Participants returned to the laboratory the following morning after an overnight fast (approximately 7-9 hours after treatment consumption). They brought with them the empty beverage container and their urine jug, which contained their first morning urine output. Subjective hunger, satiety, and desire to eat ratings were measured using a 100-mm Visual Analog Scale. Participants then consumed a standardized amount of water (8 oz). If participants needed to use the bathroom, they could do it at this time; however, they needed to use the urine jug. Thereafter, nude body weight was measured.

The performance portion of the protocol was a 10-km (6.2 miles) running treadmill TT. Participants were instructed to treat each TT as a competitive event, and accordingly provide maximal effort. Before the TT began, the participants completed a standardized warm-up at a self-selected pace. A treadmill incline of 1% was used during the TT to best simulate the oxygen cost of outdoor running. All time and speed data was blinded to the participants during testing and until the end of the study; the only known progress measure was distance covered.
Therefore, participants relied on self-pacing and were able to adjust the treadmill speed as much as desired. Participants were not allowed to use music during the TT and no encouragement was given in order to keep the testing scenarios as identical as possible.

Two designated timers kept performance time and recorded the split at every 1-km interval. HR, RPE, and running speed measurements were recorded at every 1-km interval as well. The participant again provided a complete urine sample in which total urine output and USG were measured using the same procedures as described above. The participant’s post-exercise nude weight was also measured in order to calculate changes in body mass. This allowed the researchers to determine differences in sweat loss and drink retention between CM and PLA.

**Statistical Analysis**

Power analysis using JMP Pro (SAS, Cary, NC) was conducted based on data from Ferguson-Stegall et al. (2011) and revealed the need for 12 participants. Power was set to 0.8 with an alpha of 0.05. The standard deviation (SD) was 3.28 minutes and the difference between the means was 7.94 minutes. TT endurance performance was the main variable used for analysis.

SPSS Version 21 (Chicago, IL) was used to analyze the data of this study. Two-tailed Student’s *t* test and two-way analysis of variance (ANOVA) was used to determine statistical differences between variables. All data was reported as means ± SD. Significance was accepted at *p* ≤ 0.05. The uncertainty of outcomes as 90% confidence limits (CL) and probabilistic magnitude-based inferences about the true values of outcomes by qualifying the likelihood that the true effect represents a “substantial change” are also reported. The likelihood of a substantial increase or decrease was calculated from the two-tailed Student’s *t* distribution and
was classified as follows: <0.5%, almost certainly not; 1%-5%, very unlikely; 5%-25%, unlikely; 25%-75%, possible; 75%-95%, likely; 95%-99.5%, very likely; >99.5%, almost certain. When the majority of the confidence interval (>50%) fell between the threshold for substantially positive and negative effects, the likelihood of the effect being “trivial” (negligible) was qualified\textsuperscript{84}. 
RESULTS

Participant Characteristics

A total of 22 female runners and triathletes were recruited and underwent baseline testing, with a total of 12 completing the entire protocol (Figure 3). Of the three females who were dropped after baseline testing, two did not meet the minimum VO$_2$max requirement and one was regularly taking an anti-inflammatory drug. Of the remaining seven females who did not complete the study, four dropped out due to musculoskeletal injury unrelated to the study that would have limited their performance if they were to continue, and three dropped out due to unrelated personal reasons.

Participants that completed all testing were 30 ± 6 years old and were running 30.5 ± 11.5 miles per week, with 4.2 ± 2.7 years of training experience and a VO$_2$max of 52.8 ± 4.2 ml/kg/min at the time of testing. Additional baseline data, including body composition results, are presented in Table 1.

Dietary Analysis

A total of twenty-four 3-day food logs were analyzed, one for the CM trial and one for the PLA trial. Total kilocalorie (kcal), macronutrient (carbohydrate, fat, protein (g and % of total energy)), caffeine (mg), and fluid (mL) content were analyzed for each day of the food logs. The average intake of each variable over the three days was calculated and compared between CM and PLA trials. No significant differences were found between average dietary intake for any variable between the CM trial and PLA trial (Table 2).

Subjective Appetite Measurements

Desire to eat significantly decreased the morning following CM consumption compared to PLA (45.8 ± 23.5 mm vs. 55.6 ± 22.8 mm; p=0.04). Participants’ subjective ratings of hunger decreased (CM, 41.2 ± 21.0 mm vs. PLA, 48.6 ± 25.2 mm; p=0.31) and subjective ratings of
satiety increased (CM, 44.8 ± 17.1 vs. PLA, 35.9 ± 21.8; p=0.12) following the CM compared to PLA, however, these values were not statistically significant (Table 3 and Figure 4).

**Hydration Status Measurements**

No significant differences were found between the CM trial and the PLA trial for hydration status markers (Table 4). Although not significant, the pre-10 km TT urine output (CM, 687.6 ± 261.0 vs. PLA, 826.0 ± 205.6; p=0.15) and the total urine output (CM, 718.1 ± 260.8 ml vs. PLA, 874.8 ± 179.6 ml; p=0.10) were lower for the CM trial compared to PLA. Also, the probabilistic inference revealed that the consumption of CM would likely cause a decrease in the next morning, pre-10 km TT urine output as well as the total urine output. First morning USG was not different between CM and PLA trials (1.015 ± 0.006 vs. 1.013 ± 0.007; p=0.14). The change in weight from before and after the 10-km TT was not significant (CM, 1.05 ± 0.35 kg vs. PLA, 1.07 ± 0.27 kg; p=0.91), indicating sweat rate was similar for both trials. Participants finished the TT with an identical post-10 km TT USG (1.011 ± 0.006).

**10-km Time Trial Performance**

Performance measurements were not significantly difference between the CM trial and the PLA trial (Table 5). At the halfway point (5-km), running times were not statistically different (CM, 27.3 ± 4.2 min vs. PLA, 27.3 ± 4.3 min; p=0.98). The finishing time (10-km), although not statistically different, was 3.24 seconds faster in the CM trial than the PLA trial (Figure 5). HR and RPE measurements at both the 5 km and 10 km marks were not significantly different between groups (Table 5 and Figure 6). All performance inferences were unclear or possibly trivial.
Table 1. Participant characteristics (n=12).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>30 ±6</td>
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<tr>
<td>Weight (kg)</td>
<td>58.2 ±4.4</td>
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<tr>
<td>Height (cm)</td>
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<td>Mileage (miles/week)</td>
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<tr>
<td>Training Status (y)</td>
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<tr>
<td>Body Fat (%)</td>
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<td>BMI (kg/m²)</td>
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<tr>
<td>Resting HR (bpm)</td>
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</tr>
<tr>
<td>VO2 max (ml/kg/min)</td>
<td>52.8 ±4.2</td>
</tr>
</tbody>
</table>

HR = heart rate; bpm = beats per minute
Data are mean ± SD.

Table 2. Average dietary intake over three days preceding experimental trials (n=12).

<table>
<thead>
<tr>
<th></th>
<th>CM</th>
<th>PLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Energy (kcal)</td>
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<td>3006 ±909</td>
</tr>
<tr>
<td>Total Carbohydrate (g)</td>
<td>347.3 ±118.7</td>
<td>342.5 ±100.9</td>
</tr>
<tr>
<td>Total Protein (g)</td>
<td>123.7 ±29.2</td>
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<tr>
<td>Total Fat (g)</td>
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<tr>
<td>Total Carbohydrate (%)</td>
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</tr>
<tr>
<td>Total Protein (%)</td>
<td>17.7 ±4.3</td>
<td>17.4 ±3.8</td>
</tr>
<tr>
<td>Total Fat (%)</td>
<td>34.0 ±9.4</td>
<td>35.2 ±10.2</td>
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<tr>
<td>Fluid (g)</td>
<td>2279.5 ±1120.2</td>
<td>2386.9 ±1072.3</td>
</tr>
<tr>
<td>Caffeine (mg)</td>
<td>237.7 ±329.1</td>
<td>246.3 ±368.1</td>
</tr>
</tbody>
</table>

CM = chocolate milk; PLA = placebo
Data are mean ± SD; Averages were obtained by averaging dietary intake for all 3 days prior to each experimental trial; no statistical differences were found between CM and PLA trials for any dietary component (p > 0.05)

Table 3. Visual analogue scale of hunger, satiety and desire to eat the morning after nighttime eating of CM vs. PLA (n=12).

<table>
<thead>
<tr>
<th></th>
<th>CM</th>
<th>PLA</th>
<th>P value</th>
<th>Inference++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger (mm)</td>
<td>41.2 ±21.0</td>
<td>48.6 ±25.2</td>
<td>0.31</td>
<td>Possible decrease</td>
</tr>
<tr>
<td>Satiety (mm)</td>
<td>44.8 ±17.1</td>
<td>35.9 ±21.8</td>
<td>0.12</td>
<td>Likely increase</td>
</tr>
<tr>
<td>Desire to eat (mm)</td>
<td>45.8 ±23.5</td>
<td>55.6 ±22.8</td>
<td>0.04*</td>
<td>Likely decrease</td>
</tr>
</tbody>
</table>

CM = chocolate milk; PLA = placebo
Data are mean ± SD.
++ The probabilistic inference is the chance that the true (large-sample) effect of CM will increase, will have trivial effect, or will decrease the subjective appetite ratings.
* p<0.05, between CM and PLA
### Table 4. Next morning hydration status after nighttime eating of CM vs. PLA (n=12).

<table>
<thead>
<tr>
<th></th>
<th>CM</th>
<th>PLA</th>
<th>P value</th>
<th>Inference++</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre – 10km TT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.4 ± 4.1</td>
<td>58.2 ± 4.1</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Urine Output (ml)</td>
<td>687.6 ± 261.0</td>
<td>826.0 ± 205.6</td>
<td>0.15</td>
<td>Likely decrease</td>
</tr>
<tr>
<td>USG</td>
<td>1.015 ± 0.006</td>
<td>1.013 ± 0.007</td>
<td>0.14</td>
<td>Possible trivial decrease</td>
</tr>
<tr>
<td><strong>Post – 10 km TT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.3 ± 4.2</td>
<td>57.1 ± 3.9</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Urine Output (ml)</td>
<td>30.5 ± 27.3</td>
<td>48.8 ± 53.5</td>
<td>0.29</td>
<td>Unclear</td>
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<tr>
<td>USG</td>
<td>1.011 ± 0.006</td>
<td>1.011 ± 0.006</td>
<td>0.91</td>
<td>Unclear</td>
</tr>
<tr>
<td>Total Urine Output (ml)</td>
<td>718.1 ± 260.8</td>
<td>874.8 ± 179.6</td>
<td>0.10</td>
<td>Likely decrease</td>
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<td>Change in Weight (kg)</td>
<td>1.05 ± 0.35</td>
<td>1.07 ± 0.27</td>
<td>0.91</td>
<td>Unclear</td>
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</tbody>
</table>

CM = chocolate milk; PLA = placebo; TT = time trial; USG = urine specific gravity
Data are mean ± SD.
++ The probabilistic inference is the chance that the true (large-sample) effect of CM will cause a decrease in urine output and USG, will have a trivial effect, or will cause an increase in urine output and USG. A decrease in urine output and USG indicates a superior hydration status and an increase indicates a poorer hydration status.
*p<0.05, between CM and PLA

### Table 5. 10-km TT performance variables after nighttime eating of CM vs. PLA (n=12).

<table>
<thead>
<tr>
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<tr>
<td><strong>Heart Rate</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(bpm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5km</td>
<td>171 ± 13</td>
<td>169 ± 13</td>
<td>0.28</td>
<td>Possibly trivial</td>
</tr>
<tr>
<td>10km</td>
<td>179 ± 15</td>
<td>180 ± 14</td>
<td>0.70</td>
<td>Unclear</td>
</tr>
<tr>
<td><strong>RPE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5km</td>
<td>13.3 ± 1.1</td>
<td>13.8 ± 1.4</td>
<td>0.30</td>
<td>Unclear</td>
</tr>
<tr>
<td>10km</td>
<td>15.8 ± 2.0</td>
<td>16.7 ± 2.1</td>
<td>0.20</td>
<td>Unclear</td>
</tr>
<tr>
<td><strong>Split (min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5km</td>
<td>27.3 ± 4.2</td>
<td>27.3 ± 4.3</td>
<td>0.98</td>
<td>Very likely trivial</td>
</tr>
<tr>
<td>10km</td>
<td>53.3 ± 7.9</td>
<td>53.4 ± 8.2</td>
<td>0.95</td>
<td>Very likely trivial</td>
</tr>
</tbody>
</table>

CM = chocolate milk; PLA = placebo; TT = time trial; bpm = beats per minute; RPE = rating of perceived exertion on Borg scale (6-20); bpm = beats per minute
Data are mean ± SD.
++ The probabilistic inference is the chance that the true (large-sample) effect of CM will cause a substantial decrease in performance, will have a trivial effect, or will cause an increase in performance.
*p<0.05, between CM and PLA
Figure 3. Participant flowchart.

Figure 4. Subjective appetite ratings for hunger, satiety and desire to eat the morning after CM vs. PLA consumption (n=12). Values are means. * CM significantly different from PLA (p<0.05).
Figure 5. Split time difference at the 5-km mark and 10-km mark of the TT for the CM trial. Columns represent the number of seconds faster the participants ran in the CM trial than the PLA.

Figure 6. Heart rate and rating of perceived exertion (on a 6-20 Borg scale) at 5-km and 10-km marks of the 10-km TT for CM and PLA trials (n=12). CM = chocolate milk; PLA = placebo; bpm = beats per minute.
DISCUSSION

The main findings of this study were that the nighttime consumption of CM did not improve next morning hydration status or running performance in female endurance athletes. These findings do not support the hypotheses that CM would be more effective than PLA at increasing various hydration status markers and decreasing 10-km running time. The 7-9 hour time gap between beverage consumption and exercise performance was a likely contributing factor to the lack of significant findings. However, although performance and hydration status were not altered, desire to eat was significantly lower the morning after drinking CM than PLA. This is a novel finding and for endurance athletes who must compete early in the morning, it may be beneficial to avoid hunger before short endurance races. The satiating capacity of protein, particularly dairy protein, is a probable contributor to this outcome.

Dietary Analysis

In order to maintain consistency between trials, participants were asked to track their diet and fluid intake for 3 days preceding their first experimental trial. They were given back their exact copy of the food log and asked to repeat it as exact as possible for the second trial. If they needed to deviate away from the first food log for any reason, participants took note of the differences. Each trial was analyzed separately to see if there were any differences in total energy intake, macronutrient (carbohydrate, protein, fat) intake, fluid intake, and caffeine intake. In this present study, the participants showed a high level of consistency between trials, as there were no significant differences in any measured dietary variable between the CM and PLA trial. This consistency enables researchers to isolate the nighttime beverage as the main dietary difference between the two trials.

Participants were asked to refrain from caffeine consumption the day preceding each experimental trial in order to prevent any performance enhancing capabilities that it may have. It
is evident through the food logs that participants did consume caffeine the day prior to testing. However, on average, the amount of caffeine consumed in the days prior to CM trial vs. PLA trial was not significantly different (237.73 ± 326.06 mg vs. 246.34 ± 368.06 mg, p > 0.05).

**Subjective Appetite Measurements**

The only significant value that was observed for the subjective appetite measurements was in the “desire to eat” category. Participants had a significantly lower desire to eat the next morning after the consumption of CM than they did with the PLA. Additionally, although the values were not statistically significant, the participants rated themselves as being less hungry and more satiated the morning after drinking the CM. Previous research in acute settings (15-270 minutes post-prandial) demonstrated that dietary proteins have a high satiating capacity. It has been suggested that proteins stimulate the satiety GI hormones (CCK and peptide YY) to a greater extent than other nutrients, leading to decreased hunger and less desire to eat for a longer period of time after protein is consumed. Additionally, data show casein protein to be more satiating for a longer period of time when compared to whey protein, as casein is digested at a much slower rate. This indicates why milk may have long-term satiating capacity that would be beneficial in a nighttime eating scenario, as milk is composed of 80% casein protein and 20% whey protein.

The findings in the present study are consistent with previous nighttime eating research, which may indicate that the satiating capacity of a small, protein-containing nighttime meal is sustained throughout the night. Although not statistically significant, Madzima et al (2013) also reported that next morning feelings of fullness (satiety) were greater after consuming protein the night before as compared to just carbohydrate. Also, Kinsey et al (2014) found that nighttime macronutrient intake increased satiety (p = 0.03) and reduced desire to eat (p= 0.006) the following morning.
The potential for nighttime consumption of CM to decrease the next morning desire to eat and increase satiety may be a positive factor for the endurance athlete who must compete early in the morning. It may be difficult for an athlete to eat in close proximity to their competition time without causing gastrointestinal distress. When competitions are very early in the morning, the amount of time that the athlete has to eat is limited, so therefore, athletes may choose to not eat anything, even if their hunger cues are telling them to do otherwise. If drinking CM the night before a race can decrease the magnitude of these hunger cues, the athlete will be able to focus less on wanting to eat and more on competing to the best of their ability.

**Hydration Status Measurements**

Adequate hydration status plays a major role in the outcome of an endurance athlete’s performance. Running performance has shown to decrease significantly when athletes compete in a dehydrated state as compared to a hydrated state. Previous research suggests that various components of milk (fluid, electrolytes, and protein) allow it to serve as a substantial hydration beverage. The consumption of milk in close proximity to exercise has resulted in a greater fluid retention, higher whole body fluid balance, and more optimal urine osmolality than both water and carbohydrate-electrolyte beverages.

The present study was the first of its nature, investigating two new elements of the hydrating capacity of milk; first as a pre-exercise hydration beverage as opposed to post-exercise, and second, in an overnight scenario. Our results indicate that the hydrating capacity of milk may not sustain itself throughout the nighttime hours. CM did not prove to be superior to the PLA for next morning USG, urine output, or body weight changes throughout exercise.

The results of the present study were in agreement with previous research when looking at total urine output. It was hypothesized that the total urine output the morning after beverage
consumption would be lower after the CM. Although not statistically significant, urine output was lower for the CM trial compared to the PLA trial at all measured time points (Pre-10-km TT, Post-10-km TT, and Total). Furthermore, the probabilistic inference revealed that the true effect would have caused a likely decrease (90.5%) in urine production post-CM consumption should the sample have been larger. By producing less urine, the participants retained more fluid from the CM than the PLA overnight. This lower urine production did not result in a more optimal hydration status after a 10-km TT; however, it might have enabled the athlete to maintain their hydrated state longer in the scenario of a lengthier race. This notion warrants further attention in future studies.

When looking at body weight changes, the amount of fluid retention was almost identical between PLA and CM. Previously, James et al reported that the protein content of milk allowed for a greater drink retention than that of a commercially available carbohydrate solution and water. This setting, however, was immediately post-exercise and not 7-9 hours preceding exercise. Additionally, the inclusion of milk protein in a hydration beverage has caused an increase in osmotic pressure, which in turn leads to increased water retention. Due to these known facts, it was hypothesized that the nighttime consumption of CM would allow for a greater fluid retention during exercise; however, the 7-9 hour gap between protein consumption and exercise performance may have negated these properties.

Contrary to the hypotheses, USG did not vary between CM and PLA for the first morning output or post-exercise output. Similar to USG, urine osmolality tests the level of urine dilution. When given skimmed CM as a post-exercise rehydration beverage, endurance athletes exhibited a greater urine osmolality (better hydration status) 2-3 hours post-exercise than they did after consuming water or a carbohydrate beverage. This effect can be attributed to an increase in
plasma amino acids after consuming the protein, which would exert an increased osmotic effect\textsuperscript{78}. In the present study, the CM was not used as a post-exercise hydration beverage, but rather as a pre-exercise hydration beverage. Again, the fact that the participants did not exhibit a more optimal USG with the CM consumption may be due to the fact that the urine was tested 7-9 hours post-consumption vs. 2-3 hours post-consumption like the previous studies. Should the milk have been consumed closer to the time of exercise, they athletes may have began exercise with a more optimal USG and they may have maintained a more optimal USG throughout the TT. Further research is required to investigate this idea.

A positive fact to take note of is that in both scenarios, the CM trial and the PLA trial, participants woke up the next morning with an “adequately hydrated” USG, per the American College of Sports Medicine (ACSM) guidelines\textsuperscript{42}. The average first-morning USG was 1.015 after consuming the CM and 1.013 after PLA. According to the ACSM, an individual with a first morning USG ≤ 1.020 is considered to be “euhydrated” or “adequately hydrated”\textsuperscript{42}. The closer the USG number is to 1.000 (which is the specific gravity of water), the more hydrated the individual is. Any first-morning USG between 1.020-1.030 is considered dehydrated. If the participants did not consume the extra serving of fluid prior to sleep the night before competition, this state of adequate hydration upon waking may not have been the case in the present study. However, in order to fully make this conclusion, an extra treatment would need to be added where the participants do not consume any type of fluid before sleep.

**10-km Time Trial Performance**

The last area of interest is the running performance in the 10-km TT following the nighttime eating of CM and PLA. CM and other milk-based beverages have long been a widely used recovery strategy from endurance exercise\textsuperscript{3,5–7,10–12,29}. The main goal of workout recovery
nutrition is to replenish muscle glycogen, rebuild damaged muscle, and replenish lost fluid. Well-researched sports nutrition guidelines have determined that between a 3:1 and 4:1 carbohydrate to protein ratio within 30 minutes after a workout will elicit the best glycogen and muscle protein resynthesis. Milk naturally contains this carbohydrate to protein ratio in the form of lactose sugar and casein and whey proteins. It is also high sodium and potassium. Both of these key electrolytes are naturally lost in sweat, so therefore, it is necessary to replenish them following a workout in order to properly rehydrate.

Thomas et al. (2009) demonstrated the unique properties of CM by using it as both a recovery and performance nutrition strategy. Endurance cyclists completed glycogen-depleting exercise, followed by a 4-hour recovery period during which they consumed CM, CR, or FR. The recovery period was followed up with an endurance capacity test. Performance in the endurance capacity test significantly improved following the consumption of CM.

Karp et al. (2006) also compared the efficacy of low-fat CM to carbohydrate-only and fluid replacement (containing electrolytes) beverages as a recovery aid following an initial trial of glycogen depleting exercise on a cycle ergometer. Efficacy was based on subsequent performance in a cycling TTE test. TTE was enhanced by 49% and 54% for the CM and fluid replacement as compared to the carbohydrate-only beverage, with no statistically significant differences between CM and fluid-replacement. This shows that CM is just as effective as the commercially available sports drink at enhancing further endurance capacity. Additional studies have shown similar results.

The present study was the first of its nature to use CM as a performance beverage in the nighttime eating scenario. As evidenced by the 5-km split and the 10-km finish time, the CM did not significantly improve performance time. Unlike the previously mentioned studies, the
performance bout was not preceded by a glycogen-depleting workout and the CM was consumed the night prior as opposed to only 4 hours before performance. A nighttime dose of casein protein has shown to increase the rate of MPS and net protein balance following resistance exercise, ultimately, improving recovery overnight\textsuperscript{14,16}. CM may have revealed a more positive performance enhancement the next morning should the female participants have had to complete an additional bout of exercise prior to the nighttime eating. Future studies should investigate this potential overnight recovery ability of CM with endurance athletes, as it is quite common for an athlete to have races on back-to-back days.

Although statistical differences did not occur, participants did complete the 10-km TT 3.24 seconds faster in the CM trial than in the PLA trial. In an actual race setting, 3.24 seconds can make a substantial difference in final placement of an endurance race. Also, at the 5-km mark, participants were only 0.54 seconds faster with the CM, but by the finish (10-km mark) that gap increased by 6-fold to 3.24 seconds. If the race would have been longer, the CM may have provided adequate energy for the athletes to continue to increase their speed and lengthen that gap even more so (Figure 4).

**Limitations**

As this study was the first of its nature, the current literature base posed significant limitations. Endurance performance research is very limited within the female population. The foundation of this study was primarily based on research conducted on male athletes. The genetic nature of males and females is highly variable, so therefore females may not respond the same in a performance enhancement setting. Additionally, all nighttime eating research to date has focused on male athletes and has looked at recovery from exercise as opposed to performance
the next day. Future research should continue to build within the female population in order to lessen this limitation in the future.

Limitations within the research design inevitably occurred as well. First, although training status of the athletes was well controlled for by requiring a minimum number of miles per week and a minimum VO$_2$max value, the type of endurance athlete was not controlled for. There was a good mix of athletes who were specifically runners and others who were triathletes. Training just for running as opposed to training to race three different endurance events in one race can have an impact of how the athletes approach their race. In future studies, the population should be narrowed to just triathletes or just runners to control for this barrier.

Next, the researchers did not standardize the diet and fluid intakes leading up to the performance trials. Participants were allowed to choose their own pre-performance diets, which allowed for a wide range of dietary compositions amongst participants. The negative impact of this limitation was minimized by the use of food logs and asking participants to repeat their exact diet for the second trial. However, the diet logs were self-reported. Participants may not have accurately recorded on their logs and they may not have repeated themselves as identically and the logs indicated. Future studies should consider using a standardized diet that is administered to participants by the researchers. This will allow for complete diet standardization and will isolate the nighttime beverage to an even greater extent.

In conjunction with the self-selected diet, participants were also allowed to self-select their exercise regimens on the days leading up to the experimental trials. Researchers asked the participants to minimize strenuous activity the day prior in order to avoid fatigue on the day of the TT. Also, participants were requested to complete the same exercise regimen leading up to the second trial as they did leading up to the first. Again, the exercise reports were based
completely on participant honor. Although the exercise was recorded, the researchers cannot be 100% sure that participants followed the guidelines.

Additionally, accurate consumption of the nighttime beverage was based completely on participant loyalty. All participants were given clear instructions multiple times on when to consume the beverage, however, it is only by participant honor that researchers truly know they complied. One may consider administering the beverage in the lab at the proper time to ensure compliance. Unfortunately, this might disrupt sleep patterns, which could be more detrimental to the study results. In attempt to counteract this limitation and to minimize compliance issues, we collected empty beverage containers the following morning.

The last limitation to look at is the menstrual cycle. Since hormone testing and analysis was only available in a post hoc setting, the phase of the cycle during testing was a complete estimation. All women reported the starting date of their most recent menstrual cycle, and researchers estimated the luteal phase by counting out days 15-28 of the cycle. An issue with counting is that not all women have the same cycle length. Counting out the days allows a fairly decent estimate of the phases, however, it does not ensure that all women were in the luteal phase during testing. If the women were in a different phase during testing, this could have had an impact on hydration markers and performance results.

Conclusions

In conclusion, this study is the first to assess the effects of nighttime eating of CM on hydration status and running performance in female endurance athletes. It was established that nighttime (pre-sleep) consumption of CM reduces next-morning desire to eat and does not impair 10-km TT performance in female endurance athletes. Our hypotheses were not supported, as the CM did not improve running performance, next morning hydration status, or post-exercise
hydration status when compared to the non-nutritive PLA. Nevertheless, the fact that nighttime eating of CM decreased the desire to eat the following morning may be a positive element for endurance athletes. This feature will allow the athlete to focus more on their performance and less on their hunger cues before beginning a race. Also, after consuming a nighttime beverage, athletes woke up adequately hydrated (USG of ≤ 1.020) and remained hydrated throughout the 10-km TT. The nighttime beverage did not have any detrimental effects, so it may be a useful solution to help athletes meet their nutritional and hydration needs for race morning, as well as for overall health.
APPENDIX A

IRB APPROVAL LETTER AND APPLICATION

The Florida State University
Office of the Vice President For Research
Human Subjects Committee
Tallahassee, Florida 32306-2742
(850) 644-8673, FAX (850) 644-4392

APPROVAL MEMORANDUM

Date: 4/30/2014

To: Michael Ormsbee [ormsbee@fsu.edu]

Address: 1493
Dept.: NUTRITION FOOD AND EXERCISE SCIENCES

From: Thomas L. Jacobson, Chair

Re: Use of Human Subjects in Research
Effect of Nighttime Feeding on Morning Performance in Female Endurance Athletes

The application that you submitted to this office in regard to the use of human subjects in the research proposal referenced above has been reviewed by the Human Subjects Committee at its meeting on 04/09/2014. Your project was approved by the Committee.

The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals, which may be required.

If you submitted a proposed consent form with your application, the approved stamped consent form is attached to this approval notice. Only the stamped version of the consent form may be used in recruiting research subjects.

If the project has not been completed by 4/8/2015 you must request a renewal of approval for continuation of the project. As a courtesy, a renewal notice will be sent to you prior to your expiration date; however, it is your responsibility as the Principal Investigator to timely request renewal of your approval from the Committee.

You are advised that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report, in writing, any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the Chair of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols as often as needed to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

This institution has an Assurance on file with the Office for Human Research Protection. The Assurance Number is FWA00000168/IRB number IRB00000446.

Cc: Armindeni Bahram, Chair
HSC No. 2014.12300
Human Subjects Application For Full IRB and Expedited Exempt Review

1. Project Title and Identification

1.1 Project Title

Effect of Nighttime Feeding on Morning Performance in Female Endurance Athletes

Project is: Thesis

1.2 Principal Investigator (PI)

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<tr>
<th>Name (Last name, First name MI):</th>
<th>Ormsbee, Michael James</th>
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1.3 Co-Investigators/Research Staff

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<tr>
<th>Name (Last name, First name MI):</th>
<th>Gorman, Katherine Anne; Co-Investigator</th>
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<tr>
<td>Email:</td>
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APPENDIX B

FSU HUMAN SUBJECTS COMMITTEE APPROVED INFORMED CONSENT

Effect of Nighttime Feeding on Morning Performance in Female Endurance Athletes

Informed Consent Form

1. I voluntarily and without element of force or coercion, consent to participate in the research project entitled “Effect of Nighttime Feeding on Morning Performance in Female Endurance Athletes.” This study is being conducted by Dr. Michael Ormsbee, Katherine Gorman and Elizabeth Miller within the Department of Nutrition, Food, & Exercise Sciences at Florida State University.

2. The purpose of the proposed study is to determine the influence of nighttime feeding on next morning running performance, hydration status, and exercise metabolism in female endurance athletes. Twelve moderately trained females between the ages of 18-45 will be recruited for this study. ‘Moderately trained’ will be defined as a weekly running mileage of at least 25 miles for at least the last 6 months. Additionally, qualification for this study will be dependent on the attainment of a relative maximal oxygen consumption ($VO_{2\max}$) value greater than or equal to 40.0 ml/kg/min. $VO_{2\max}$ represents the maximal ability of the body to utilize oxygen and is representative of cardiopulmonary fitness.

3. My participation in this study will require coming to the Human Performance Laboratory at The Florida State University for a $VO_{2\max}$ test and three trials: one familiarization trial, and two experimental trials. Each trial will comprise approximately 90 minutes. Additionally, I will be required to briefly visit the laboratory the day prior to each experimental trial to pick up a treatment beverage and urine collection container, and provide a saliva sample. The experimental trials will be completed within a 2-week period that is determined by the luteal phase of the menstrual cycle (days 15-28 of the menstrual cycle, with day 1 being the first day of menstruation)². A minimum of 48-72 hours will be provided between testing days.

On the first visit, I will arrive to the laboratory in the morning, fasted (overnight fast), and complete the informed consent paperwork, medical history questionnaire and specific endurance-training questionnaire. Initial height and weight measurements will be completed using a Physician’s Scale with attached height apparatus (Seca, Mexico). Both measurements will be taken barefoot and in minimal exercise apparel. My body composition will be determined by measurement of body density through air displacement technique (BOD POD; COSMED).

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Initials

Effect of Nighttime Feeding on Morning Performance in Female Endurance Athletes

I will then complete a VO2max test using a graded treadmill (Woodway PPS Med; Waukesha, WI) and metabolic cart system (Parvo Medics TrueMax 2400 Metabolic Measurement System; Consensus Technologies; Sandy, UT) to ensure qualification for the study and to establish running intensities for an incremental exercise test (IET) portion of the performance protocol. I will be fitted with a nose clip and headpiece, including a mouthpiece with breathing tube attachment to collect expired air and deliver to cart. I will be fitted with a Polar™ heart rate monitor. The testing protocol will allow me to select a pace that is ‘comfortable, but challenging’ for the duration of the test. Once an appropriate speed is determined, it will be held constant, while the grade is increased at a rate of 2% every 2 minutes. Heart rate (HR) and rating of perceived exertion (RPE), measured on a 6-20 Borg scale, will be recorded during the last 15 seconds of each stage. The test will be terminated when I can no longer keep pace. My maximal exercise intensity will be defined by the velocity and grade of the last completed stage.

I will not be eligible to participate in this study if my VO2max value is less than 40.0 ml/kg/min. I will not be eligible to participate in this study if I am lactose intolerant, currently smoke (or have quit within less than 6 months), have an irregular or absent menstrual cycle, have an uncontrolled thyroid condition, regularly take anti-inflammatory drugs or any dietary supplements to improve performance, or have a musculoskeletal injury that could limit my running performance.

By meeting these criteria, I will qualify for participation in this study and receive further instruction on details regarding upcoming experimental trials. I will be required to complete a 3-day dietary food and exercise log prior to the first experimental trial. It is important that I replicate my recorded food intake and exercise habits exactly prior to the subsequent trials. I will also be instructed to abstain from use of non-steroidal anti-inflammatory drugs, caffeine, and/or participation in vigorous activity for at least 24 hours prior to each experimental trial.

My second visit to the laboratory will serve as a familiarization to the experimental exercise trials. All details of the exercise trials are explained below. I will not receive a treatment beverage or undergo blood or urine testing or provide a saliva sample during the familiarization trial; however, all other specifications will be exactly the same as the experimental trials.

Over the span of the study, I will receive the following treatments: a performance beverage and placebo. Beverages will be provided in identical opaque containers; empty containers will be collected in effort to verify compliance.

Initials

Effect of Nighttime Feeding on Morning Performance in Female Endurance Athletes

Visit 3 will occur the day prior to the first experimental trial. I will be instructed to come to the lab to obtain the designated treatment beverage and a urine collection container for use the following morning. I will be required to drink the treatment beverage at least 2 hours after my last meal and within 30 minutes prior to sleep. During this visit to the lab, I will also provide a small saliva sample by passively drooling into a collection container. Prior to providing the sample, I will rinse my mouth with water. This saliva sample will be used to test my progesterone hormone level, which will verify whether I am in the luteal phase of the menstrual cycle.

Visit 4 will require that I return to the laboratory the following morning after an overnight fast (approximately 7-9 hours after treatment consumption). Prior to arrival at the laboratory, I will be instructed to completely empty my bladder into the given collection container. Should I need to urinate additionally after arrival to the laboratory but prior to exercise, I will be required to use the collection container, as my total urine output will be measured. In addition, 1 mL of my collected urine will be used to measure urine specific gravity (USG), a hydration index, by a digital hand-held refractometer (ATAGO® Pocket PAL-105).

After urine data are collected, I will be provided 8 oz (240 mL) of water for consumption prior to testing. I should not consume any food or beverage prior to this time. Thereafter, my body weight will be measured according to the same procedure from Visit 1. I will then sit for approximately 15 minutes for collection of resting metabolic data. Data from the last 5 minutes of collection will be used for analysis. Resting HR and finger-stick blood sample (~0.5 mL) to determine glucose and lactate values will be collected at this time. Blood measures will be taken via finger-stick, using lancet and heparinized capillary tube, and will be analyzed immediately. My subjective satiety rating will be measured using a 100-mm Visual Analog Scale.

After resting measurements, the mask will be removed and I will complete a 5-minute warm up at a self-selected pace. Following the warm up, I will complete the incremental exercise test (IET) portion of the performance test. To analyze substrate use, the mask will be replaced to collect respiratory exchange ratio (RER) data via the metabolic cart while I complete 5-minute stages at incremental exercise intensity (65%, 75%, 85% velocity of VO_{2max} determined from VO_{2max} testing). Treadmill speed and grade data for each IET stage will be calculated from data at maximal exercise intensity; the accuracy of derived values will be confirmed during testing and may be adjusted slightly to most accurately

Initials

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represent the respective percentage. HR and RPE will be recorded during the last 15 seconds of each stage. Additionally, a finger-stick blood sample for glucose and lactate analysis will be collected at the end of each stage. Upon completion of the IET, my body weight will be measured a second time, this time nude with dry skin in the presence of female researchers only. Total time between The IET and the subsequent time trial will comprise approximately 10 minutes.

As mentioned, the next portion of the protocol will be a 10-kilometer (6.2 miles) time trial (TT). I must treat all TTs as a competitive event, and accordingly provide maximal effort. A treadmill incline of 1% will be assigned during the time trial to best simulate the oxygen cost of outdoor running. I will be blinded from all time and speed data during testing and until the end of the study; my only known progress measure will be distance covered. Therefore, I must rely on self-pacing and will be able to adjust the treadmill speed as much as I desire. TT performance will be recorded by 3 designated timers. HR and RPE measurements will be taken at every 1-kilometer interval. At the 5-kilometer point, I will be instructed to momentarily straddle the treadmill belt for a finger-stick blood sample (~15 seconds).

Additional blood samples will be taken via the same finger-stick method both immediately- and 10-minutes post-exercise. All blood samples will be analyzed immediately for glucose and lactate. My post-exercise nude weight will be measured to calculate whole body net fluid balance (calculated from changes in body weight). I will again be instructed to provide a urine sample in which total urine output and USG will be measured.

Visits 5 and 6 will be identical to visits 3 and 4, respectively. During visit 5, I will receive the treatment beverage that I did not receive prior to the first performance trial (visit 3). All of the previous instructions regarding Visits 3 and 4 will apply to Visits 5 and 6. The performance trials will be separated by 48-72 hours. My testing will be complete after Visit 6.

4. If I agree to participate in this study, I understand there is a minimal amount of risk involved. All protocols have been previously used in related studies and qualified personnel will be present during all experimental trials to ensure that proper procedures are followed. I may experience muscle soreness and fatigue related to multiple bouts of maximum-effort running. Risks associated with VO2max testing include temporary muscle aches, joint pain, and general fatigue both during and following the test. Although extremely rare, there is a minimal risk of serious musculoskeletal injury or other conditions, such as sudden cardiac events (e.g. heart attack or chest pain) or breathing complications occurring.

__________

Initials

Effect of Nighttime Feeding on Morning Performance in Female Endurance Athletes

during testing. The risk of injury and cardiovascular events during the tests will be minimized by careful review of my medical history form. My previous training questionnaire will be analyzed to determine that my current training has prepared me for the caliber of these tests. If the information provided on my medical history and endurance training questionnaires does not warrant safe participation, I will not be allowed to participate in this study.

The risks from blood draw via finger-prick are small; however, there may be some local discomfort at the puncture site. The risk of local infection is also minimal. These risks will be minimized by the presence of skilled technicians and the use of sterile techniques and equipment. The risk of adverse events from consumption of the performance beverage is extremely minimal.

5. The possible benefits of my participation in this research project include knowledge about my body composition, \( VO_{2\text{max}} \), hydration status markers, and resting and submaximal exercise metabolism measures. Additionally, I may potentially benefit from a nutritional strategy to enhance competitive endurance performance.

6. The results of this study may be published but my name or identity will not be revealed. Information obtained during the course of the study will remain confidential to the extent required by law. My name will not appear on any of the results. No individual responses will be reported in publication, but group responses. Confidentiality will be maintained by the assignment of a code number for each subject in which all data record will be based. The only record containing both the participant’s name and code number will be kept by the principal investigator, Dr. Michael Ormsbee, in a locked drawer in his office. All records will be destroyed after a minimum of three years.

7. If I become injured during testing, first aid (free of charge) will be provided to me by the laboratory personnel working on the research project. However, any additional treatment required will be provided at my own expense.

8. Any questions I have concerning this research study or my participation, before or after my consent, will be answered by the investigators or referred knowledgeable source. I understand that I may contact Dr. Michael Ormsbee at [removed], Katie Gorman at [removed], or Beth Miller at [removed] for answers to questions.

Initials

Effect of Nighttime Feeding on Morning Performance in Female Endurance Athletes

about this research study or my rights. Group results will be sent to me upon my request after the completion of the study.

9. In case of an injury, or if I have questions about my rights as a participant in this research, or I feel I have been placed at risk, I can contact the chair of the Human Subjects Committee, Institutional Review Board, through the office of the Vice President of Research at (850) 644-8633 (humansubjects@fsu.edu).

10. The nature, demands, benefits and risks of the study have been explained to me. I knowingly assume any risk involved.

11. I have read the above informed consent form. I understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of the benefits to which I may otherwise be entitled. In signing this consent form, I am not waiving my legal claims, rights or remedies. A copy of this consent form will be given to me.

Print Name

Signature Date

Initials

APPENDIX C

HEALTH HISTORY QUESTIONNAIRE

Date: _________________________________                       ID#: _________

HEALTH HISTORY QUESTIONNAIRE

The following questions are designed to obtain an understanding of your medical history. The information you provide will allow researchers to make an accurate determination about your eligibility to participate in this current study. Please answer all questions to the best of your ability and provide as much information as possible. This questionnaire, as well as all other medical information you provide will be kept confidential and will not be shared with unauthorized personnel or organizations unless you specifically request the researchers to do so.

Name: __________________________________________________________________

Street Address: ___________________________________________________________

City, State, Zip code: ______________________________________________________

Telephone Number:  H (       )___________________  C (       )_________________

Email Address: ___________________________________________________________

Date of Birth (mm/dd/yy): ______________________ Age: _______________________

Sex:    M ______ F ______

Personal Physician’s Name: _______________________ Phone: (       )______________

Address: ________________________________________________________________

Height: _______ in _______ cm       Weight: _______ lb _______ kg
Current Occupation: ______________________________________________________

Race: __________________________________________________________________

Date: _________________________________                       ID#: _________

**Personal Health History**

Have you ever been hospitalized or had surgery? Yes _______ No ________

Please list all hospitalizations and surgeries to the best of your recollection

<table>
<thead>
<tr>
<th>Hospitalized for</th>
<th>Disease/Operation</th>
<th>Duration</th>
<th>Age when hospitalized</th>
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</table>

List any disease or illness you have not listed above (e.g. mumps, measles, broken bones, etc.)

Are you allergic, sensitive, or intolerant of any foods (e.g. soy, wheat, shellfish, grain, milk, etc.)? ) or medications?

Yes _____    No ______

If yes, please describe:

   Food: ____________________________________________________________
Medication: _______________________________________________________
Other: ____________________________________________________________

Are you currently seeing a doctor or other health care provider for any reason?
Yes _____  No _____
If yes, please explain:

Date: _________________________________                       ID#: _________

Do you have any musculoskeletal injuries or other health problems that may impair your running performance? Please explain:

Do you have any neurological problems including fainting, dizziness, headaches, or seizures? Please explain:

Does anyone in your family (immediate family including your grandparents) have a history of cardiovascular disease (heart attacks, stroke, etc.)? Please explain:

Do you smoke or use smokeless tobacco? Yes _____ No ______
Have you smoked within the last 3 months? Yes _____ No ______
Do you drink coffee or other caffeinated beverages? Yes _____ No ______
If yes, what kind, how much, and how often?

Please list all vitamins, minerals and herbs, and other nutritional (performance) supplements as well as medications you are currently taking. How long have you been taking them and how frequently?

<table>
<thead>
<tr>
<th>Supplement/Medication</th>
<th>Duration</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

Are you willing to stop taking all nutritional supplements you are currently using for the duration of this research study? Yes _____ No ______

Date: _________________________________                       ID#: _________

How would you describe the type of diet you currently eat? Have you recently been on any special diets? What kinds of diets have you used to lose weight or lower cholesterol? Please list and describe:

What changes, if any, have you made to your diet in the last 6 months?

**Female Specific History**
Do you have a regular menstrual cycle? Yes _____ No _____

What was the date of your last period (first day)? ________________________________
   (See attached calendar for help)

Are you currently taking birth control medication? Yes_____ No_______
   If yes, how long have you been taking birth control? _______________________
   If yes, what is the name of the specific medication? ________________________

Has a doctor ever told you that you are anemic (low iron)?  Yes _____ No ______
   If yes, did you supplement with iron? ___________________________________
   Do you currently supplement with iron (may be listed previously in the supplements
   section)? _______________________________

Has a doctor ever told you that you have low bone density?  Yes _____ No ______

Has a doctor ever told you that you are underweight?  Yes _____ No ______

__________________________________________  ___________________________
Participant Signature                   Date
ENDURANCE TRAINING QUESTIONNAIRE

The following questions are designed to obtain an understanding of your endurance training history. The information you provide will allow researchers to make an accurate determination about your eligibility to participate in this current study. Please answer all questions to the best of your ability and provide as much information as possible. This questionnaire will be kept confidential and will not be shared with unauthorized personnel or organizations unless you specifically request the researchers to do so.

On average, how many miles do you run per week? _________________

On average, how many days per week do you run? _________________

How many months/years have you been training at or near this level? ________________

If you participate in other endurance exercise (swimming, cycling, triathlon etc), approximate your hours per week of training outside of running ________________

How often do you race (running) competitively? ________________________________

What distances do you typically race? _________________________________________
   If you have raced the 10-kilometer distance, what is your personal best? ______
   In what time do you think you could race this distance currently? _____________
Do you participate in resistance training? If yes, please describe:

_______________________________    __________________
Participant Signature

__________________________  __________________
Date
APPENDIX E

THREE-DAY FOOD LOG

Date: _________________________________                       ID#: _________

3-DAY FOOD AND ACTIVITY RECORD

Please fill out the following food and activity logs to the best of your ability. Please write down everything you eat and drink or any exercise you complete for the 3 days leading up to your first experimental trial. It is important that you repeat this exact food and drink routine for the 3 days before each of your trials for this entire research study. Please bring the completed forms to your first experimental trial.

Food Recall
Record what you have eaten as soon as possible after meals. This makes it much easier to remember what and how much you eat. Remember the following:

- **Preparation:** How was the food cooked? Was it grilled, fried, steamed, or baked? Was it fresh, frozen or canned?

- **Portion size:** Indicate how much of each food you eat by using cups, ounces, teaspoons, or tablespoons, or a handful where possible. For meats, estimate the ounces you eat. (The size of deck of cards or a computer mouse is about a 3-ounce portion). See the attached sheet with portion size estimations.

- **Include the fluids that you drink.** List the amounts and the types, and the times that you drink them.

- **Include the extras or condiments you eat:** Do you put cream or sugar in coffee? Is your tea sweetened or unsweetened? Do you use ketchup, mustard, mayonnaise, steak sauce, or salsa on foods?

- **Be specific:** If you eat bread, is it white, wheat, whole wheat, rye, honey wheat or multigrain? If you drink milk, it is whole, 2%, 1%, skim, soy, or rice milk? Etc. Include brand names or labels from food items when possible.

- **Record only one food item per line:** If you eat a salad with several components (lettuce, tomato, cheese etc), write each component out separately.

- **Restaurant eating:** If you eat at a restaurant, do your best to estimate portion size and list the name of the restaurant. List any visible fat, oil, or sauces added to your food.
Exercise/Activity Recall
Record any exercise or daily activities you participate in. Be specific in terms of duration and intensity

![Estimation of Portion Sizes](image)

- 3 oz (75 g) cooked chicken or meat (4 oz raw) – deck of cards
- about 3-4 oz meat – palm of your hand
- 1 cup (250 ml) cooked rice, pasta, or ice cream – tennis ball
- medium piece of fruit – baseball
- 1 tsp (5 ml) butter or margarine – one die
- 1 tsp – knuckle to tip of thumb
- 1 cup (250 ml) – average woman’s fist 1/2 cup - small handful
- 2 tbsp (30 ml) peanut butter, jam, salad dressing – golf ball
- 1 small baked potato – a computer mouse
- 1 oz (30 g) of chocolate – a packet of dental floss
- 1 oz (30 g) cheese – 4 dice or 1 domino
**EXAMPLE**

<table>
<thead>
<tr>
<th>Time of day</th>
<th>Serving size</th>
<th>Food item</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:30 am</td>
<td>1 cup</td>
<td>Cheerios</td>
<td>Sat on the couch</td>
</tr>
<tr>
<td></td>
<td>½ cup</td>
<td>2% milk</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 cup</td>
<td>Apple juice</td>
<td></td>
</tr>
<tr>
<td>8:30 am</td>
<td></td>
<td></td>
<td>Recovery run (4 miles around 8:30 pace)</td>
</tr>
<tr>
<td>10:00 am</td>
<td>1 medium</td>
<td>Banana</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 cup</td>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>12:00 pm</td>
<td>2 slices</td>
<td>McDonald’s Bread – hamburger bun</td>
<td>Walked short distance to and from class</td>
</tr>
<tr>
<td></td>
<td>1 slice</td>
<td>Cheddar cheese</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 patty</td>
<td>Hamburger</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 supersized</td>
<td>French fries</td>
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<tr>
<td></td>
<td>1 16 ounce</td>
<td>Regular coke</td>
<td></td>
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<tr>
<td>3:30 pm</td>
<td>15</td>
<td>Crackers - Sociables</td>
<td>Worked at desk (seated)</td>
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<tr>
<td></td>
<td>2 Tbsp</td>
<td>Peanut butter</td>
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<td></td>
<td>1 8 ounce</td>
<td>Juice box</td>
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<tr>
<td>6:30 pm</td>
<td>5 ounces</td>
<td>Chicken –thigh - baked</td>
<td>Watched TV</td>
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<tr>
<td></td>
<td>1 ½ cups</td>
<td>rice</td>
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<td></td>
<td>½ cup</td>
<td>Broccoli</td>
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<td></td>
<td>1 cup</td>
<td>2% milk</td>
<td></td>
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<tr>
<td></td>
<td>½ cup</td>
<td>Mixed fruit – fruit cocktail with sauce</td>
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<tr>
<td>7:45 pm</td>
<td>1 ½ cups</td>
<td>Vanilla ice cream</td>
<td>Watched TV</td>
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<tr>
<td></td>
<td>3 Tbsp</td>
<td>Chocolate sauce</td>
<td></td>
</tr>
<tr>
<td>8:30 pm</td>
<td></td>
<td></td>
<td>Did house chores</td>
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</table>

**Do not forget to record beverages, including water**

Was this a fairly typical day for you in terms of food intake and exercise? Explain

*No, this was not a typical day’s intake because I had a doctor’s appointment and went to McDonald’s afterwards for lunch, but my exercise/activity was fairly normal.*
DAY 1

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<th>Specific activity</th>
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** Do not forget to record beverages, including water

Was this a fairly typical day for you in terms of food intake and exercise? Explain
Date: _________________________________                       ID#: _________

DAY 2

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<th>Time of day</th>
<th>Serving size</th>
<th>Food item</th>
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** Do not forget to record beverages, including water

Was this a fairly typical day for you in terms of food intake and exercise? Explain
Date: _________________________________                       ID#: _________

DAY 3

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<th>Food item</th>
<th>Specific activity</th>
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</table>

** TAKE YOUR NIGHTTIME BEVERAGE (record above)**

** Do not forget to record beverages, including water

Was this a fairly typical day for you in terms of food intake and exercise? Explain
REFERENCES


57. Davis BA, Thigpen LK, Hornsby JH, Green JM, Coates TE, O’Neal EK. Hydration kinetics and 10-km outdoor running performance following 75% versus 150% between bout fluid replacement. *Eur. J. Sport Sci.* 2014;0(0):1–8.


BIOGRAPHICAL SKETCH

Elizabeth Miller received her Bachelor of Science degree in Food, Nutrition, and Dietetics from Tennessee Technological University in May 2013. While at Tennessee Tech, Elizabeth was the member of the Varsity Cross Country and Track teams on which she served as captain her senior year. Elizabeth presented her undergraduate honors research project entitled “Effects of Nutrition Education on Dietary Perceptions and Habits of College Distance Runners” at two professional conferences and won the 2013 TTU Research Day award. After TTU, she began graduate studies at Florida State University where she is currently completing the requirements for the combined Master’s of Science in Exercise Science with a major in Sports Nutrition and Dietetic Internship program. Elizabeth is working under the supervision of Dr. Michael Ormsbee in the Department of Nutrition, Food, and Exercise Science in the College of Human Sciences. She is a graduate teaching assistant for Sports and Exercise Nutrition and is a Sports Nutrition Intern for the FSU Athletics Sports Nutrition Department. Upon completion of her degree in May 2015, Elizabeth will continue on to a career as a Registered Sports Dietitian in the collegiate athletic setting. In her free time, Elizabeth enjoys running, hiking, cooking, spending time outdoors, being involved in her church community and relaxing with her friends and family.