Effects of Nighttime Feeding on Exercise Metabolism and Performance in Female Endurance Athletes

Katherine Anne Gorman
EFFECTS OF NIGHTTIME FEEDING ON EXERCISE METABOLISM AND PERFORMANCE IN FEMALE ENDURANCE ATHLETES

By

KATHERINE ANNE GORMAN

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The members of the supervisory committee were:

Michael J. Ormsbee
Professor Directing Thesis

Lynn B. Panton
Committee Member

Robert J. Contreras
Committee Member

The Graduate School has verified and approved the above-named committee members, and certifies that the thesis has been approved in accordance with university requirements.
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BACKGROUND: Nutrient timing is an effective means of augmenting endurance exercise training and performance. Previous studies report that pre-exercise feeding can influence exercise metabolism up to 4 hours post-prandial. However, this timeline has been confirmed during waking hours only; little is known about how sleep within the post-prandial period may influence metabolism during subsequent exercise. This question is relevant to the endurance competitor, as race start-times often occur in early morning, limiting the opportunity for optimal feeding prior to competition without disrupting sleep or risking gastrointestinal distress.

PURPOSE: To investigate the influence of a small, nutrient dense, pre-sleep chocolate milk (CM) beverage on morning metabolism and 10-km running performance in female athletes.

METHODS: In a crossover design, twelve competitive female runners (age, 30 ± 7 yrs; VO\textsubscript{2peak}, 53 ± 4 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) ingested either pre-sleep CM or a non-nutritive, flavor-matched placebo (PL) ~30 min before sleep and 7-9 hrs before a morning performance running trial. Following initial appetite assessment (visual analogue scales) and resting metabolic rate (RMR), serum glucose (GLU) and lactate (LAC) measurements, the performance trial included a warm-up and three 5-min incremental loads at 55, 65, and 75% VO\textsubscript{2peak} (to measure respiratory exchange ratio (RER), GLU and LAC), followed by a 10-km treadmill time trial (TT). Paired t-tests, ±90% confidence intervals, and magnitude-based inferences were used to determine differences in means. Significance was accepted at $P < 0.05$. RESULTS: Relative to PL, CM showed a ‘likely small decrease’ in perceived hunger ($P = 0.041$, –22.3% mean effect), and a ‘likely small increase’ in RMR ($P = 0.049$, 4.8% mean effect), resting GLU ($P = 0.081$, 3.7% mean effect), and RER at 55, 65, and 75% VO\textsubscript{2peak} ($P = 0.115$, 0.194, 0.164 and 2.1%, 1.6%, 1.4% mean effect, respectively). Exercise GLU revealed subtle, and unique trends following CM compared to PL.
Specifically, GLU was ‘possibly increased’ during exercise at 65% VO\textsubscript{2peak} \((P = 0.358, \ 2.0\%\ \text{mean \ effect})\) and ‘likely trivially decreased’ at 75% VO\textsubscript{2peak} \((P = 0.561, \ -1.0\%\ \text{mean \ effect})\) following CM compared to PL. No differences in resting or exercise LAC, or 10-km TT performance \((\text{PL: } 52.8 \pm 8.4 \text{ mins versus CM: } 52.8 \pm 8.0 \text{ mins, } P = 0.987, \ -0.1\%\ \text{mean \ effect, 'most likely trivial' decrement to performance following CM compared to PL})\) were noted between treatments. **CONCLUSIONS:** Nighttime supplementation of CM results in acute enhancement to morning metabolism via increased carbohydrate utilization during exercise, but has no apparent effects on 10-km running performance. These results suggest that the nighttime feeding timeline, specifically sleep imposed within the post-prandial period may extend tangible effects to metabolism to over 8 hours out from meal ingestion.
CHAPTER 1

INTRODUCTION

Fueling for optimal performance has become an important component of the training program for endurance athletes. As training techniques become more advanced, sport-specific nutritional strategies are often sought to gain a performance advantage. Nutrient timing is one approach to maximize training quality, recovery, and ultimately performance. While much research has focused on nutrient timing in close proximity to training (pre-, during-, post-training) (3, 13, 14, 31, 39, 51, 59, 60, 63, 72, 79, 81, 82, 113, 124), evidence is beginning to mount that suggests nighttime pre-sleep nutrient timing may also be beneficial for active individuals (84, 107). It is therefore logical to theorize that this strategy may also improve exercise performance.

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 to competition without disrupting normal sleep patterns or risking gastrointestinal (GI) distress. For this reason, many endurance athletes do not eat much, if anything, before competitions of less than 60 minutes in duration; reiterated by recommendations from the most recent International Olympic Committee (IOC) position statement for sport nutrition (89). Unfortunately, even for endurance athletes competing for 60 minutes or less, those who compete following an overnight fast may begin with significantly depleted liver glycogen stores (95) and with the missed opportunity to optimize exercise metabolism (1, 11, 92, 108) and skeletal muscle protein (PRO) balance (29, 39, 70, 82). The incorporation of a pre-sleep meal may provide an added window of opportunity to establish an optimal pre-race physiological condition that would otherwise be missed.
Nighttime consumption of a carbohydrate-protein (CHO+PRO) beverage, specifically chocolate milk (CM), 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 CHO+PRO beverage makes for an effective post-endurance exercise recovery aid (39, 72, 79, 82, 117, 121, 124, 134). There is also evidence to suggest that CM is an effective nutritional strategy before and during endurance exercise (3, 59). CM generally provides adequate carbohydrate (CHO) and PRO (casein, whey, and branch chain amino acids) to address system needs before, during, and most notably, after exercise (3, 39, 59, 72, 79, 82, 117, 121, 124, 134). As there is preliminary evidence to suggest that CHO, casein, and whey PRO are efficiently metabolized at night (41, 49, 84, 107), CM may be an effective supplement for the nighttime feeding scenario.

Despite the practicality and potential benefits of eating before sleep for morning endurance performance, little data exist at present. While outcomes from potential nighttime feeding research may be advantageous the athletic population as a whole, the notoriously under-researched female athletic population may benefit specifically. The emphasis of the female athletic population in such research may serve a dual-role: to demonstrate female-specific performance improvements, and arguably more important, to attenuate the disparity between genders in the realm of exercise performance research.

Therefore, the purpose of the present study was to investigate the effects of acute pre-sleep supplementation of CM versus a non-nutritive placebo (PL) on morning 10-kilometer (km) running performance and measures of exercise metabolism in female endurance athletes. It was hypothesized that pre-sleep CM would enhance exercise metabolism for short-duration, high-intensity endurance-type exercise, and thus improve performance compared to PL.
Specific Aims

1. To determine changes in morning running performance in response to acute pre-sleep CM in female endurance athletes as measured by time to complete a 10-km treadmill time trial (TT) protocol (Woodway PPS Med; Waukesha, WI).

2. To determine changes in morning exercise metabolism in response to acute pre-sleep CM evaluate in female endurance athletes as measured by:
   a. Respiratory exchange ratio (RER) data collected during incremental exercise (55, 65, 75% peak oxygen consumption (VO$_{2peak}$)) via metabolic cart systems (Parvo Medics Truemax 2400 Metabolic Measurement System; Consentius Technologies; Sandy, UT).
   b. Blood glucose and lactate data collected and analyzed during incremental exercise (55, 65, 75% VO$_{2peak}$) and at two post-TT time points via finger stick blood draw and hand-held analyzers (OneTouch UltraMini® Blood Glucose Meter; LifeScan, Inc.; Milpitas, CA; and Lactate Plus; Nova Biomedical Corp.; Waltham, MA).

Research Hypotheses

1. Pre-sleep CM will result in enhanced 10-km TT performance via a decreased time to completion versus PL.

2. Pre-sleep CM will result in an increased reliance on CHO oxidation during exercise, as indicated by an increased RER. Blood glucose will be maintained to a greater extent following CM treatment compared to PL. Blood lactate will be higher during exercise with CM compared to PL.
Assumptions

1. Subjects consumed 100% of their treatment beverage (CM/PL).
2. Subjects consumed their treatment beverage (CM/PL) at the correct time point as directed by researchers.
3. Subjects put forth maximal effort during VO₂peak testing and all exercise trials.
4. Subjects completed health history, endurance training questionnaire and food and exercise logs (3-day) truthfully and to the best of their ability.
5. All laboratory equipment was utilized properly by researchers and provides accurate results.
6. Subjects followed all instructions provided by researchers.

Delimitations

1. This study included a total of 12 females who were considered moderately trained endurance runners.
2. Trained status correlated with a relative VO₂peak value ≥ 45.0 ml·kg⁻¹·min⁻¹ and a consistent weekly running mileage (≥ 25 miles) for at least 6 months prior to participation in the study.
3. Those individuals who did not consistently use oral contraceptives (greater than 2 months) or who were not considered eumenorrheic without oral contraceptive use were excluded to minimize hormonal differences among subjects.
4. Those individuals with lactose-intolerance were excluded.
5. Those individuals with musculoskeletal injury that would limit performance were excluded.
6. Those individuals taking nutritional supplements intended to improve performance at or near the time of data collection were excluded.

**Limitations**

1. The inability to control subjects’ diet, exercise habits, and supplementation adherence outside of testing sessions.

2. The sensitivity of the performance protocol to detect differences between treatment groups.

3. The potential that CM treatment beverage and flavor-matched PL were not identical in flavor or texture.

**Definitions of Terms**

**Branched-chain amino acids (BCAAs)** – component of whey protein that is a potent trigger for protein synthesis, suppression of protein catabolism, as well as gluconeogenesis; leucine in particular is a BCAA critical in stimulating muscle protein synthesis (53)

**Casein protein** – the major component of bovine milk (approximately 80%); casein is characterized by slow digestion and absorption (53)

**Essential amino acids (EAAs)** – amino acids that cannot be synthesized by the body and must therefore be provided by the diet (109)

**Fasted** – a period of at least 12 hours in which no food or beverage is consumed (91)

**Fatigue** – in relation to endurance exercise, when workload can no longer be maintained due to substrate depletion or increased skeletal muscle pH (21)

**Competitive or high-intensity endurance exercise** – repetitive, aerobic exercise completed at an intensity ≥ 70% of VO$_{2\text{peak}}$ (110)
**Muscle protein balance** – the net relationship between skeletal muscle protein synthesis and skeletal muscle protein breakdown (109)

**Muscle protein synthesis (MPS)** – the accretion or repair of skeletal muscle tissue; of particular emphasis is the mammalian target of rapamycin, or mTOR pathway (109, 137)

**Nighttime feeding** – a small, calorically dense (~150 kcal) meal provided in the hours after dinner (~2 hours) and immediately prior to sleep (~30 min) (41, 74, 84)

**Peak oxygen consumption (VO$_{2peak}$)** – the peak ability of the body to utilize oxygen; may be represented in absolute (l·min$^{-1}$) or relative (ml·kg$^{-1}$·min$^{-1}$) terms (21)

**Performance** – in relation to endurance exercise, an increase in sustainable workload over a period of time, an increase time to volitional fatigue (time to exhaustion), or a reduction in time to complete a standard distance (time trial) (65)

**Prolonged endurance exercise** – repetitive, aerobic exercise completed for duration ≥ 2 hours (31)

**Respiratory exchange ratio (RER)** – a measure of relative fat and carbohydrate substrate utilization during exercise as determined by the ratio VCO$_2$/VO$_2$ of gas exchange measures; the measurement continuum includes 0.7 (fat-only oxidation) to 1.0 (carbohydrate-only oxidation) (11)

**Resting metabolic rate (RMR)** – the energy expenditure required by the human body at rest, typically measured indirectly by gas exchange (84)

**Short-duration endurance exercise** – repetitive, aerobic exercise for duration < 60 min (64)

**Whey protein** – the liquid protein component of bovine milk (approximately 20%); whey is characterized by rapid digestion and absorption (53)
CHAPTER 2

REVIEW OF LITERATURE

Nighttime Feeding

Population-Specific Research. Nighttime feeding is a relatively new phenomenon in the arena of sports nutrition. Most original research in this area stems from nighttime shift workers (83) or those with nighttime-eating disorders (47, 87) with primarily negative outcomes for cardiovascular disease risk factors and body composition. However, these outcomes may be 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 (83). Low meal frequency is associated with low resting metabolic rate (RMR) and nighttime consumption of high calorie (~700 kcal) and non-nutrient dense meals (47). Thus, the overall diet and quality of a nighttime meal may dictate potential outcomes more greatly than the presence of a nighttime meal alone.

Clinical Research. This notion is evidenced by more recent research that suggests physiological benefits from nighttime feeding in populations that are not shift-workers (41, 49, 84, 107, 133). In these controlled studies, where treatment meals were small (~150-200 kcals), nutrient dense, and composed primarily of a single macronutrient, outcomes were positive. The data include a broad range of health outcomes (41, 49, 74, 133). Combined with an exercise intervention, Figueroa et al. (2014) reported improvements to systolic blood pressure and arterial stiffness with nighttime feeding of milk PRO in overweight and obese sedentary hypertensive females (41). Besides potential direct benefits to arterial health and function, there is preliminary evidence to suggest that nighttime feeding may also improve body composition in clinical populations, presenting an additional and independent pathway to ultimately reduce cardiovascular disease (CVD) risk. Kinsey et al. (2014) examined the acute effects of nighttime
eating in overweight and obese women and reported improvement to next morning appetite measures (subjective satiety and desire to eat) with nighttime feeding of whey and casein PRO, and CHO (74). However, insulin resistance was negatively affected (74), suggesting that the sedentary overweight and obese population may be ‘metabolically insensitive’ to a nighttime feeding of PRO or CHO, as next morning insulin levels were augmented with all treatments. In general, obese individuals tend to have higher fasting insulin values than lean individuals (33), perhaps suggesting a propensity for the development of unfavorable metabolic adaptations in this population independent of nighttime feeding. Additionally, the absence of an exercise intervention in this study, a well-regarded mechanism to enhance insulin sensitivity (18), may have also contributed to unfavorable outcomes. Interestingly, this negative effect was completely reversed when carried out for four weeks with an exercise program (3 days per week) (99). However, the aforementioned acute satiety outcomes from this study may offer a partial explanation for the major and positive findings from another, longer duration study on nighttime feeding of cereal and low-fat milk (133).

Waller et al. (2004) reported reduced energy intake and increased weight loss when overweight subjects consumed a nighttime cereal snack versus no nighttime meal (133), providing evidence for improved body composition with a nighttime feeding intervention in overweight or obese sedentary populations. Interestingly, nighttime feeding of PRO may also improve body composition by increasing lean mass, particularly in the clinical elderly population (49). As aging is characterized by the progressive decline in skeletal muscle size and function (76), nutritional interventions have been designed to attenuate losses. Groen et al. (2012) examined nighttime ingestion of casein PRO and reported increases in muscle PRO synthesis (MPS) rates and whole body PRO balance versus non-caloric placebo in elderly males (49).
Outcomes from this study are valuable in that they offer a separate and additional strategy to enhance body composition in clinical populations.

**Translation to Exercise Performance.** Those outcomes discussed among recent nighttime feeding research in clinical populations may also be applicable to a healthy, physically active population (84, 107). Specifically, Madzima et al. (2013) examined the effect of acute nighttime supplementation of various PRO and CHO treatment beverages on next morning measures of RMR in healthy, young, physically active males (84). It was found that regardless of substrate (casein PRO, whey PRO, or CHO), there was an increase in RMR compared to non-caloric placebo. Additionally, a follow-up study by Res et al. (2012) reported similar results to the original, Groen et al. (2012), with nighttime PRO ingestion yielding increased MPS rates and whole body PRO balance in a younger, resistance trained population (107). Taken together, these data demonstrate that not only can PRO be digested and metabolized normally during sleep, but also that it directly impacts MPS and potentially recovery from exercise. As nighttime feeding shows health benefits, including body composition and metabolic health in several populations, it is also logical to theorize that nighttime feeding may improve exercise performance.

However, as with all research in this area, 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 (84, 107). 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 (44, 123). Similarly, the fact that PRO can be absorbed effectively at night and promotes MPS (107) has
relevant application for recovery from exercise both acutely (125) and habitually (109). Additionally, most nighttime meals provided in the previous research, specifically that from Madzima et al. (2013) and Kinsey et al. (2014), have had a composition similar to most post- and/or pre-exercise meal recommendations (150-300 g CHO with moderate PRO content; post-exercise meal often recommended based on body weight) (60, 61, 92, 118), lending further theoretical application to exercise performance research. Moreover, the nighttime beverage proposed in the present study contains both CHO and whey and casein PRO, which have each independently elicited positive results with nighttime consumption in previous research (41, 49, 74, 84, 107).

While not directly related to the study in question, outcomes from Tsuchida et al. (2013) may be potentially valuable for endurance performance. Major applicable findings from this research include the consumption of a nighttime meal, ‘late night supper’ (2300 hrs) resulting in increased efficiency in next morning CHO digestion and absorption versus traditional timing of an evening meal (1800 hrs) (127). If these findings translate to an increased efficiency of CHO oxidation, this specific modification to CHO digestion and absorption kinetics could have major benefit to certain endurance competition scenarios. For example, this would be the case for late-day endurance competition (i.e. allowing ample time for a pre-race meal), and/or prolonged endurance competition (≥ 2 hours) in which exogenous CHO feeding is recommended for optimal performance, as maximal benefit is dependent upon rate of absorption and subsequent oxidation (31, 110). This research suggests further potential for nighttime feeding in regards to next day endurance performance, which could perhaps be considered for future research. However, as the present study is characterized by early-morning competition less than 2 hours in length, inferential value from Tsuchida et al. (2013) is purely speculation.
High-Intensity Endurance Performance Determinants

The main physiological factors that can affect endurance performance include substrate availability and metabolism, muscle PRO balance, and hormonal influence, particularly for female athletes.

Substrate Availability and Metabolism. Endurance exercise relies on adequate energy turnover in the form of adenosine triphosphate (ATP) in order to support metabolism and muscle contraction. Stored fat and CHO comprise the main energy sources for ATP production during endurance exercise; the relative contribution of each being dependent on exercise intensity and duration (31, 111). Low- to moderate-intensity exercise, defined as ≤ 60% of peak, or maximal oxygen consumption (VO$_{2\text{max}}$) is supported primarily by fat metabolism, while high-intensity (> 70% VO$_{2\text{max}}$) substrate utilization will shift more towards CHO (111). As most competitive endurance exercise is completed at an intensity greater than 70% VO$_{2\text{max}}$, the athlete relies most often on available CHO in the form of stored muscle and liver glycogen, and blood glucose (110). Long-term training will induce a relative increase in fat metabolism at a given submaximal exercise intensity (103), thought to be mediated by increased mitochondrial biogenesis (37) and presence of oxidative enzymes at the skeletal muscle (62), an adaptation demonstrated in both males and females (27). In general, females tend to have greater reliance on fat metabolism than their male counterpart, as indicated by a lower RER at the same relative exercise intensities (27). However, at intensities greater than 70%, substrate utilization seems to be similar across genders.

It is well established that exercise endurance capacity is reduced when blood glucose is low and muscle glycogen stores are depleted (12, 31, 120, 132). Classic research by Bergstrom et al. (1967) first demonstrated this phenomenon following the manipulation of CHO content in the diets of endurance-trained male cyclists. Not only did a high-CHO diet (approximately 82%
CHO; 2,800 kcal; 2,300 kcal CHO; 500 kcal PRO) translate to higher muscle glycogen content, but also to increased cycling time-to-exhaustion (TTE) (70% VO$_{2\text{max}}$), when compared to a mixed-diet (uncontrolled) (12). Research throughout the last half-century confirms the relationship between dietary CHO-supplementation, pre-exercise muscle glycogen stores, and subsequently fatigue resistance (100, 120). Importantly, these results have also been demonstrated in female endurance athletes, suggesting that strategies to enhance glycogen storage are just as critical to female exercise performance (132). Besides augmenting endogenous CHO storage, research has evolved to include exogenous feeding of CHO during prolonged endurance exercise as an effective performance strategy. Coyle et al. (1986) was one of the first to demonstrate the ergogenic effect of CHO feeding during exercise. Although glycogen utilization was unaffected by CHO supplementation, CHO oxidation was maintained late in exercise likely as a result of exogenous CHO oxidation (31). Several publications since confirm the performance enhancement of CHO feeding during prolonged high-intensity endurance performance (30, 88, 90, 126).

As it pertains to the current study, it is generally regarded that high-intensity exercise performance lasting less than 2 hours is not limited by glycogen storage, as muscle and liver stores are thought to be adequate (95). However, this notion presupposes that athletes are in a fed state. In the early morning, fasted state, glycogen levels may not be sufficient, even for shorter duration high-intensity exercise (95). This claim is further evidenced by recent literature that has demonstrated exogenous CHO consumption during high-intensity exercise ≤ 60 minutes to be advantageous, although these effects may be related to non-metabolic mechanisms (i.e. central fatigue) (64). Thus, even for shorter duration exercise, a nighttime meal may be warranted to at least attenuate any overnight losses to liver glycogen in order to begin
competition with adequate CHO availability even when an athlete decides to compete without a pre-exercise meal.

The effect of a pre-race meal on subsequent exercise metabolism has also been investigated as an alternative strategy to augment glycogen stores and potentially manipulate substrate utilization during exercise. In relation to the current study, the effects of a CHO+PRO treatment should be examined specifically, as it is most closely related to the composition of the CM treatment beverage. Bergman et al. (1999) examined the effect of nutritional status (i.e. fed versus fasted) on relative substrate metabolism in a subsequent exercise bout (11). Trained male cyclists were either provided a standardized pre-exercise meal (550 kcal: 87% CHO, 2% fat, 11% PRO) 3 hours prior to an exercise trial or began exercise following a 12-hour fast. At intensity $\leq 59\%$ VO$_{2\text{max}}$, fed subjects exhibited an increased RER, reflective of an increased dependence on CHO oxidation compared to fasted subjects (11). However, at greater intensities (80% VO$_{2\text{max}}$), RER was dictated more by workload than nutritional intervention (11). Surprisingly, blood lactate values were not different between groups (11). Outcomes suggest that at lower intensities a pre-exercise meal may have greater influence on RER. These findings seem logical; however, how they relate to a nighttime meal specifically in the female athletic population is yet to be investigated. As females tend to rely on fat metabolism to a greater extent (27), perhaps this population will be more sensitive to the metabolic effects of a pre-exercise meal.

Research from non-athletic populations suggests other potential benefits from a pre-exercise CHO+PRO meal. Roberts et al. (2013) examined meal composition and subsequent changes to resting metabolism in healthy, untrained males and females (108). The addition of PRO to a CHO meal produced a lower glycemic response compared to CHO-only despite an
increased serum insulin response (108). This is notable for several reasons. First, as other research suggests that the rate of muscle glycogen synthesis stemming from a meal is at least partially dependent on serum insulin concentrations (80, 138), the addition of PRO to a CHO supplement may enhance glycogen storage. Second, a hyperinsulinemic response stemming from a pre-exercise meal has been associated with a dramatic drop in blood glucose with the commencement of exercise sometimes resulting in what is known as rebound hypoglycemia (66). Roberts et al. (2013) reported that despite a higher insulin response, glycemia may be better maintained with CHO+PRO versus CHO-only. Altogether, it seems logical that adding PRO to a CHO supplement may both enhance glycogen storage and maintain euglycemia during exercise via this observed insulinemic response (60, 80, 138). It should be noted that a hyperinsulinemic response has been associated with an acceleration of glycogen breakdown as well as inhibited fat oxidation (43, 75). However, these findings are reported in studies utilizing a high CHO meal given within 1-2 hours prior to exercise and may be blunted in the nighttime feeding scenario with a CHO+PRO beverage.

**Muscle Protein Balance.** In order to support skeletal muscle growth and recovery, it is critical for muscle to remain in positive net PRO balance, determined by the rate of MPS compared to the rate of breakdown. As skeletal muscle comprises approximately 40% of total body PRO stores, exercise has a particularly relevant influence on PRO balance (109). In particular, chronic endurance exercise appears to accelerate muscle PRO turnover (104). Pikosky et al. (2006) reported an increased rate of muscle PRO turnover (increased synthesis and increased breakdown), and ultimately a more negative net muscle PRO balance following 4 weeks of aerobic exercise training (104). As dietary PRO is a major supplier of plasma essential amino acids necessary for the building and repair of muscle tissue, it is important for endurance
athletes to maintain appropriate dietary PRO intake to minimize the risk of a negative PRO balance (17) and perhaps decreases to performance (26). However, the specific influence of exercise type, intensity, or duration, as well as training status on muscle PRO turnover magnitude and direction is not entirely elucidated, therefore recommendations for dietary supplementation are less clear (109).

As it pertains to the present study, CM provides both casein and whey PRO. Tipton et al. (2004) reports that both milk PROs are sufficient to increase plasma essential amino acids and ultimately stimulate MPS to maintain in positive PRO balance (125). From a chronic recovery standpoint, an increased intake of PRO mediated in part by an additional nighttime meal of CM would be advantageous. However, as the nature of this study is acute, less emphasis may be placed on the role of PRO in regards to muscle recovery, and more rather on its role in maximizing muscle glycogen synthesis. However, this role is valid and may be considered for future, specifically long-term research.

**Ergogenic Effects of Chocolate Milk**

Consumption of milk-based beverages has long been a proposed recovery strategy for endurance exercise (39, 72, 79, 82, 121, 124, 134). 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 (79) or arguably enhanced (39, 82, 117, 124) status as a post-exercise recovery aid compared to the traditional commercial CHO+PRO performance beverage.

The effects of milk, or a comparable CHO+PRO beverage supplementation, on endurance capacity has been investigated. Karp et al. (2006) compared the efficacy of low-fat CM to CHO-only replacement 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 (70% VO$_{2\text{max}}$). Results were favorable to CM, with enhanced TTE versus CHO-only (40.0 ± 14.7 min vs. 26.8 ± 10.3 min, respectively). However, CM and CHO treatments were matched for CHO content only meaning that CM had a higher overall caloric content (72). Therefore, it is unclear whether the results were due to the specific macronutrient components or simply the additional calories of the CM beverage (72). Thomas et al. (2008) completed a similar study using treatments matched for caloric content. Cycling TTE (70% max power) was still improved to a greater extent with CM compared to CHO-only (51% longer cycle time vs. CHO-only). These results are mirrored in more recent literature (39, 82). However, not all research supports an enhanced exercise capacity with a similar CHO+PRO beverage. Betts et al. (2007) demonstrated that a comparable CHO+PRO beverage did not elicit increased running TTE (70% VO$_{2\text{max}}$) when compared to a CHO-only treatment (13). However, it is worth noting that while performance outcomes were not different between treatments, the CHO+PRO treatment was at least comparable to the traditional beverage and did not lead to any detriment in performance (13, 79). 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 CHO+PRO beverage fed prior and during a bout of endurance performance. Alghannam (2011) studied the effects of a CHO+PRO beverage compared to an isocaloric CHO beverage supplemented before and in between intermittent bouts of soccer specific, glycogen depleting exercise. It was concluded that the CHO+PRO treatment increased run time to fatigue (80% VO$_{2\text{peak}}$) and total distance covered compared to CHO alone (3). Ivy et al. (2003) demonstrated similar results in a prolonged and variable intensity cycling trial with supplementation of either a CHO+PRO or
CHO-only beverage just prior and throughout testing. There was a 36% improvement in cycling TTE (85% VO$_{2\text{max}}$); however, treatments were matched only for CHO content, not calories (59). Conversely, Lee et al. (2008) saw no differences in cycle TTE (70% VO$_{2\text{peak}}$) between a low-fat milk and a CHO-electrolyte beverage given prior and during testing (79). Although no apparent benefits were reported using the milk treatment, performance was not hindered, and compares to that of the CHO-electrolyte beverage.

In the nighttime feeding scenario, CM 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. Although rates of glycogen and PRO synthesis would not likely be accelerated in this case, as in the instance of immediate post-exercise consumption (61), synthesis is still favorable (15, 16, 40, 102). Most importantly, research has shown that, when fed prior to sleep, this normal rate of synthesis, at least for casein PRO, is maintained during the nighttime (improved versus placebo) (107). Additionally, there is evidence that a mixed macronutrient nighttime meal may even effect next day absorption of CHO, and in the case of the athlete, maximize utilization of CHO typically fed before and/or during exercise (127). Lastly, beverages of similar nature to milk have been shown to be effective prior and during exercise (3, 59), 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 kcals), nutrient dense, and primarily CHO+PRO meal such as milk might be of particular benefit to an endurance athlete the morning after nighttime supplementation.
Proposed Mechanisms of Chocolate Milk

**Carbohydrate Content.** CM contains amounts of CHO comparable to most other commercially available performance beverages (116). Thus, this serving provides an adequate bolus for muscle glycogen synthesis and blood glucose maintenance beneficial to several exercise scenarios, as well as a source for habitual CHO consumption (116). In particular, a 180 kcal or 12 oz (355 ml) serving of skimmed CM contains approximately 30 g CHO. In regular milk, CHO is primarily in the form of lactose, while CM is comprised of 50% lactose, with the remaining CHO in the form of sucrose (116). Most other recovery beverages contain primarily glucose or maltodextrin as a primary CHO source (116). It has been speculated that the different CHO make-up between CM and other recovery beverages could be the reasoning for enhanced performance with a CM beverage (124). Although, some research suggests the opposite, claiming that the different CHO composition between CM and the other treatments could be the cause for decreased performance outcomes within the CM condition (79). When lactose is digested, it is quickly hydrolyzed to its constituent monosaccharides, glucose and galactose, via the enzyme lactase (63). 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 (63). It seems that sucrose, however, is digested at rates similar to glucose (63). Slower absorption of exogenous CHO, 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 CHO composition of milk is in the case of the lactose-intolerant portion of the athletic population.

**Protein Content.** CM has a high biological value, meaning that it contains several EAAs, provided by the whole PROs: casein and whey (53). In particular, a 180 kcal or 12 oz (355 ml)
serving of skimmed CM contains approximately 12 g PRO. The ratio of casein and whey PRO 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 (125). Increased availability is advantageous to increased MPS (15). In the athletic spectrum, the addition of PRO to recovery treatment has more often than not, resulted in positive PRO balance (29, 39, 70, 82). One proposed mechanism in the post-exercise state is enhanced messenger RNA and associated mammalian target of rapamycin (mTOR) signaling, resulting in greater MPS, and net positive PRO balance (39, 82). Specifically, the mTOR signaling pathway initiates a series of phosphorylations and thus, activations of several downstream intracellular target PROs; this cascade will eventually lead to an increased translation of muscle PROs via messenger RNA (36). What is especially important is that both insulin and EAAs (specifically leucine) are thought to activate the mTOR pathway independently (36). As CM contains both EAAs, and CHO and PRO to mediate an insulin response, it is suggestive that there may be a dual-mechanism to enhance MPS (36). Thus, CM seems to be particularly advantageous to MPS post-exercise. The fact that the digestion and absorption of PRO remains intact during sleep potentiates a possible ‘untapped’ opportunity for additional MPS, which is of particular advantage to the athlete (49, 107).

Likewise, it has been suggested that the addition of PRO to a CHO recovery beverage may augment glycogen synthesis via greater blood insulin levels (14). Given the nighttime feeding scenario, the presence of PRO and CHO in milk could substantiate gains in overnight MPS and glycogen synthesis.

Furthermore, PRO in CM may attenuate muscle PRO damage resultant from exercise (81), however not all studies support this finding (39). Whey PRO in particular contains several branch chain amino acids (BCAAs) (53, 116), and besides primary use as a substrate in MPS,
similar to EAAs, BCAAs also suppress PRO catabolism (53). Luden et al. (2007) studied the effects of a CHO+PRO recovery beverage on plasma creatine kinase (CK) levels, an indirect measure of muscle PRO damage, in collegiate runners. It was found that those who supplemented with CHO+PRO accrued less plasma CK, versus the CHO-only intervention (81). However, Ferguson-Stegall et al. (2011) reported no such differences between a milk-based treatment and CHO-only treatment. If such mechanism transferred to the nighttime feeding window, decreased PRO 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.

**Considerations for the Female Athlete**

**Menstrual Cycle Physiology.** Often considered a challenge in the recruitment of female subjects in physiological research is the presence of the complex 28-day hormonal cycle that is apparent in normal females of reproductive age. Understanding the menstrual cycle allows the researcher to resolve this challenge and better design the study model to control for monthly hormone changes and ultimately substantiate female physiological research.

The menstrual cycle is often quantified based on the following: the first day denotes the start of menses, while the last day (typically day 28) denotes the last day before the next menses (122). The fluctuation of hormones along the hypothalamo-pituitary-ovarian endocrine axis will dictate the physiological changes that accompany the menstrual cycle each month. Specifically, the hypothalamus will episodically release gonadotropin-releasing hormone (GnRH) to stimulate the anterior pituitary gland (AP) to selectively secrete either follicle-stimulating hormone (FSH) or luteinizing hormone (LH) depending on the phase of the cycle (122). FSH or LH will then
stimulate the ovary to secrete either estrogen or progesterone, respectively. The relative concentration of these hormones will govern follicle and corpus luteum development, and in the absence of subsequent fertilization, menstruation (122) (Figure 2.1). In effect, the levels of estrogen and progesterone will modulate FSH and LH secretion at the AP or GnRH secretion at the hypothalamus (122).

Figure 2.1 The hypothalamic-pituitary-ovarian axis and negative feedback mechanisms. Adapted from Emanuele et al. (2002) (38).

The relative secretions of the pituitary and ovarian hormones over the 28-day cycle are illustrated in Figure 2.2. The first day of the cycle through approximately day 14 comprises the follicular phase. As denoted by its name, FSH is the predominant hormone of the follicular phase (122). Under the influence of FSH, several primordial follicles will begin to enlarge at the ovary. Additionally, the uterine lining will begin to progressively thicken (122). One follicle will soon become dominant and continue to grow, while the other, non-dominant follicles effectively
degenerate (122). The mature follicle will begin to produce estrogen. At approximately day 14, estrogen levels will peak and signal the AP and hypothalamus through negative feedback to relatively reduce the production of FSH and instead begin production of LH (122).

Figure 2.2 Relative secretions of pituitary and ovarian hormones and follicle development during the menstrual cycle.
Adapted from Ross & Vande Wiele (1974) (112).

The increased production of LH denotes the start of the second phase of the menstrual cycle, called the luteal phase (days 14-28). After this surge, the follicular wall of the dominant follicle will rupture to release an ovum into the peritoneal cavity, an event called ovulation (101). The crater of the ovulated follicle will form the corpus luteum, a fatty structure that will act transiently as a gland to secrete progesterone for the remainder of the luteal phase (122). The luteal phase is also characterized by an increase in basal body temperature, mediated by
progesterone (54). In the event that the ovum is not fertilized, the corpus luteum will degrade, causing a regression in progesterone production (122). Reduced levels of both estrogen and progesterone will cause a shedding of the ovum, corpus luteum, and uterine lining in an event called menstruation, marking day 1 of the next cycle (122).

**The Menstrual Cycle and Performance.** Of primary interest to the present study are any differential performance or substrate utilization effects during various phases of the menstrual cycle. Large discrepancies exist in the research as to whether the menstrual cycle phase has an impact on relative substrate utilization. Some research reports that female athletes utilize less muscle glycogen (relying more on fat oxidation) during the luteal phase as compared to the follicular phase during cycling exercise completed at \( \leq 65\% \) of \( \text{VO}_{2\text{max}} \) (34, 139). The augmented levels of progesterone in the luteal phase may be responsible for this potential shift in substrate oxidation. Progesterone may antagonize some of the ‘CHO-favorable’ actions of estrogen. For example, the estrogen mechanism to increase plasma glucose availability and uptake by type I fibers in the quadriceps is suppressed in the presence of progesterone, mediated via downregulation of the GLUT-4 transporter (25). Conversely, other research supports that estrogen may be ‘fat-favorable’ and that progesterone may subsequently have inhibitory effects to relative fat oxidation due to an increased body temperature and heart rate (54), respiration rate (10), and reduced fatty acid utilization (52) in the luteal (high progesterone) phase of the menstrual cycle. However, it appears that nutritional status is a large determinant in whether menstrual phase will influence relative substrate utilization (24). Specifically, Campbell et al. (2001) noted differences in substrate metabolism only when subjects completed exercise following an overnight fast (24).
As such, several other studies claim that the phase of the cycle has no effect on substrate oxidation during high-intensity exercise (97, 130). This research includes an 30 km cycling TT and 70% VO$_{2max}$ rowing ergometer bout, respectively, in which no changes in substrate utilization were noted between trials completed during the follicular and luteal phases (97, 130). Additionally, there were no differences in 30 km cycling TT performance outcomes within the first study (97). In both studies, exercise testing was completed in the fed state (97, 130). Similarly, though substrate utilization was not measured, Bailey et al. (2000) reported no difference in cycle TTE (70% VO$_{2max}$) between the follicular phase and luteal phases (6). The culmination of this research suggests that nutritional status, exercise intensity, or both, may be a more critical determinant of substrate metabolism during exercise than phase of the menstrual cycle.

**Oral Contraceptives and Performance.** Of further consideration is the effect of oral contraceptives (OC) use on substrate metabolism and endurance performance. OC are often employed to promote a more stable hormonal environment for females. OC use helps to regulate the length of the menstrual cycle and maintain normal hormonal fluctuations throughout its entirety (23, 50, 105). Thus, in the simplest terms, OC maintain constant levels of estrogen and progesterone throughout the 28-day cycle, though the specific amounts and fluctuations of each hormone will depend on dosage and type (22). Multiple studies have investigated the effect of OC use in general, as well as within specific phases of the OC cycle (active and non-active pills), on substrate oxidation (71, 105, 129, 130). Vaiksaar et al. (2011) studied the differences in substrate oxidation between both OC users and eumenorrheic non-users, as well as in OC users during active and non-active pill phases (1-hour at 70% VO$_{2max}$). In both models, there were no differences, either between use and non-use of OC, or particular subsets of OC users in substrate
oxidation. Importantly, other research documents no differences in actual performance outcomes between active and non-active pill phases (105).

In general, it seems that exercise fuel utilization completed at higher intensities is not impacted by the menstrual cycle phase in normal, non-OC users, though some conflict does still exist during exercise at lower intensities. Any conflict seems to be further minimized when pre-exercise nutritional status is considered. It is unclear whether a nighttime meal may also attenuate differences. In the case of OC use, it appears that there are no differences between users and non-users as well as between users during different pill phases. Thus, in the current study, both eumenorrheic non-OC users and OC-users are included.

**Nutritional Deficiencies.** Besides the complex physiology, one must also consider the unique psychology of the female athlete, specifically an increasingly apparent fixation on body weight, most often in pageantry and weight-bearing sports. While there are internal and external pressures among both genders, there is a greater tendency for female athletes to develop unhealthy nutritional restraint to maintain a perceived ‘ideal’ racing weight (9, 85). While the outcomes from the current study primarily relate to performance, pre-sleep nutrition (particularly over the long-term) may provide additional benefits in scenarios such as Relative Energy Deficiency in Sport (REDS), previously known as the Female Athlete Triad. Although outside the specific scope of current study, the use of pre-sleep nutrition could be one method utilized by healthcare professionals to correct the energy deficiency, and particularly the PRO deficiency, associated with reduced body weight, menstrual dysfunction, and ultimately REDS (85).
Summary

To our knowledge, an investigation of the effects of pre-sleep consumption of milk or CM on morning endurance performance and metabolism has not been undertaken. CM may be a useful substrate to provide an additional, low-calorie and nutrient-dense meal to the athlete just prior to sleep and in relatively close proximity to subsequent morning exercise (7-9 hours). The potential for additional glycogen synthesis, as well as an enhanced exercise metabolism are the basis for next morning performance improvements, especially in the fasted state. If effective, this strategy may be practical to eliminate the risk of gastrointestinal distress during exercise that often occurs following a pre-exercise meal, particularly among running events compared to cycling (106). For these reasons, an examination of the effects of nighttime feeding and next morning endurance running performance is warranted.
CHAPTER 3

MATERIALS AND METHODS

Subjects

Twelve moderately trained female endurance runners and triathletes (age 18-40 years) were recruited from Florida State University and the surrounding Tallahassee area to participate in the study. Subjects were recruited through personal contacts, flyers, local track and triathlon clubs, and the Florida State University Track & Field team.

Initial recruitment criteria included experience with regular running training (self-reported minimum of at least 25 miles-week\(^{-1}\) in the 6 months preceding the study) and competition. Additionally, subjects needed to possess a minimum VO\(_{2\text{peak}}\) of 45.0 ml·kg\(^{-1}\)·min\(^{-1}\). Subjects were required to undergo an initial screening to ensure meeting all inclusion criteria, including normal menstrual physiology (with or without regular OC use). In addition, those with musculoskeletal injury, lactose intolerance, uncontrolled thyroid conditions, current smoking status (within the last 3 months), or supplementing with ergogenic aids were excluded. All experimental procedures were explained verbally and in writing, including risks of participation prior to receiving subject written informed consent (Appendix A). This study received ethical approval from the Florida State University Human Subjects Institutional Review Board (Appendix B).

Research Design

The study design was a double blind randomized crossover with a minimum 72-hour washout between trials. Experimental trials were completed within a 14-day period determined by the luteal phase of the menstrual cycle (days 15-28) (101). Testing included a baseline visit
followed by three trials: one familiarization trial and two experimental trials (Figure 3.1). The familiarization served to minimize learning effects between the experimental trials.

**Figure 3.1** Research design overview.

**Baseline Testing**

Subjects reported to the laboratory at least 2 hours post-prandial and having avoided strenuous exercise in the preceding 24-hour period. Following informed consent, and completion of health history and endurance training questionnaires (Appendices C and D), height and weight measurements were taken using a physician’s scale with attached stadiometer (Seka; Mexico City, Mexico). Subjects were measured barefoot, wearing only a sports bra and athletic
shorts and were instructed to wear identical clothing for all remaining visits. Body composition was determined using air displacement plethysmography (BOD POD; COSMED).

VO$_{2\text{peak}}$ was determined using a graded treadmill (Woodway PPS Med; Waukesha, WI) exercise protocol and metabolic cart systems to measure gas exchange and ventilatory parameters (Parvo Medics Truemax 2400 Metabolic Measurement System; Consentius Technologies; Sandy, UT). Outcomes from testing were analyzed to ensure qualification for the study and to establish running intensities for an incremental exercise portion of the performance protocol. The metabolic system was calibrated prior to all testing 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$_2$) and carbon dioxide (CO$_2$) (16% O$_2$; 4% CO$_2$; Airgas Puritan Medical; Lenexa, KS).

Subjects were fitted with a nose clip and headpiece, including a mouthpiece with breathing tube attachment to collect expired air and deliver it to the cart, and a Polar$^\text{TM}$ heart rate monitor. The testing protocol allowed subjects to select their own treadmill speed described as ‘comfortable, but challenging’ to remain constant for the duration of the test. Grade was increased by 2.0% following each two-minute stage (98). Heart rate and rating of perceived exertion (RPE), measured on a 6-20 Borg scale, were recorded during the last 15 seconds of each stage. Testing was terminated at volitional exhaustion (98). Peak running velocity was estimated using the final treadmill speed and grade and the following formula: $S = S_T + (0.045 \cdot S_T) \cdot i$, where $S$ = peak speed in km·hr$^{-1}$, $S_T$ = testing treadmill speed in km·hr$^{-1}$, and $i$ = inclination as a percent (7, 21).
Familiarization and Experimental Trials

Subjects reported to the laboratory 1 day prior to each experimental trial to retrieve the designated pre-sleep beverage. Subjects were instructed to consume the beverage at least 2 hours after their last meal and within 30 minutes of sleep.

On the day of the trial (the following morning) subjects returned to the laboratory fasted (approximately 7-9 hours after treatment consumption). Subjects reported their approximate sleep time (hours) and normalcy (typical or atypical) of sleep. Subjects also estimated their perceived hunger, satiety, and desire to eat using 100-mm Visual Analogue Scales (VAS) (42). Subjects were weighed according to previous specifications and then positioned under a metabolic hood to collect resting metabolic data via indirect gas exchange (Parvo Medics Truemax 2400 Metabolic Measurement System; Consentius Technologies; Sandy, UT). All system calibration was identical to exercise testing, except standard gas concentrations (16% O₂; 1% CO₂; Airgas Puritan Medical; Lenexa, KS). Subjects’ rested supine on a sports medicine table in a dark, quiet, isolated testing room. Data were collected continuously for 15 minutes, with data from the final 5 minutes used for analysis. Resting heart rate was collected during this time. Blood sample (~0.5 ml) to measure blood glucose and lactate values were taken via finger-stick, using lancet and heparinized capillary tube, and analyzed immediately (OneTouch UltraMini® Blood Glucose Meter; LifeScan, Inc.; Milpitas, CA and Lactate Plus; Nova Biomedical Corp.; Waltham, MA).

Subjects completed a 5-minute treadmill warm up at self-selected pace. The exercise protocol followed with an incremental exercise test (IET) including three 5-minute stages at 55, 65, and 75% VO₂peak (Figure 3.2) (113). Treadmill speed and grade data for each IET stage were estimated from peak running velocity; the accuracy of derived values was confirmed and
adjusted if necessary during the familiarization trial. Substrate use was analyzed via respiratory exchange ratio (RER) data collected by the metabolic cart, fitted mouthpiece and tubing. Blood sample (~0.5 ml) collection was administered immediately following each stage (~30 second straddle of treadmill belt) for blood glucose and lactate analysis. Heart rate and RPE were recorded during the last 15 seconds of each stage. After the final stage, subjects were given approximately 10 minutes to use the restroom and stretch if desired (specific activities were noted and duplicated). Eight (8) oz (240 ml) of water was provided for consumption in a split dose: 4 oz prior to warm-up and IET and 4 oz after IET.

Figure 3.2 Timeline of events for the experimental protocol.

The performance protocol consisted of a 10-km (6.2 miles) treadmill time trial (TT). Subjects were instructed to treat each TT as a competitive event, and accordingly provide maximal effort. A treadmill incline of 1.0% was assigned to best simulate the oxygen cost of
outdoor running (69). All time and speed data were blinded to subjects during testing; the only known progress measure was distance completed. Therefore, subjects relied entirely on self-pacing and were able to adjust treadmill speed as often as desired. Performance times were recorded using two synchronized digital stopwatches. Additionally, split times were recorded for each km. Heart rate and RPE measurements were taken at each km. Blood sample (~0.5 ml) collection was administered immediately- and 10-minutes post-exercise for blood glucose and lactate analysis.

All laboratory testing was completed in a controlled laboratory setting (22 ± 1°C, 50 ± 10% relative humidity) (Vantage VUE™; Davis Instruments; Hayward, CA). To further reduce thermal stress, a standing floor fan was placed at a fixed speed and distance from the treadmill. Note: Subjects did not receive treatment beverages or undergo blood collection during the familiarization trial; however, all other specifications in the protocol were identical.

Treatments

Subjects received the following treatment beverages over the span of two trials: CM (TruMoo® Fat Free Chocolate Milk; TruMoo Milk; El Paso, TX) (140) and flavor-matched, non-nutritive PL (Dymatize Enterprises LLC; Bedford, TX) (Table 3.1).

<table>
<thead>
<tr>
<th>Table 3.1 Nutritional content of pre-sleep beverages.</th>
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<tbody>
<tr>
<td>Drink content</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Volume (ml)</td>
</tr>
<tr>
<td>Energy (kcal)</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
</tr>
<tr>
<td>Protein (g)</td>
</tr>
<tr>
<td>Fat (g)</td>
</tr>
<tr>
<td>Sodium (mg)</td>
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<tr>
<td>Potassium (mg)</td>
</tr>
</tbody>
</table>

Note: Data are raw means.
The volume of CM was calculated to provide appropriate amounts of CHO and PRO, and similar calorie content to beverages previously reported in nighttime feeding research. Beverages were provided in identical opaque containers. Empty containers were collected to verify compliance. A random number generator was used to produce a treatment-testing schedule (random.org).

**Dietary and Exercise Controls**

Subjects were instructed to maintain consistent dietary and exercise habits for the 72 hours preceding each trial. Subjects completed a food and exercise log preceding the first trial, and were instructed to replicate the record exactly prior to the second trial (Appendix E). Subjects were additionally instructed to abstain from non-steroidal anti-inflammatory drug and caffeine use, and vigorous activity for at least 24 hours prior to each trial. The Food Processor Version 10.13.1 dietary software was later used for dietary analysis (ESHA Research; Salem, OR).

**Statistical Analysis**

Required sample size \((N = 12)\) was estimated by power analysis (JMP Statistical software; Cary, NC) and the following predefined parameters: power of 0.8, alpha-level \((\alpha)\) of 0.05, standard deviation (SD) of \(\pm 4.6\%\), and difference to detect of 8.1% carbohydrate utilization during exercise (4).

Ninety percent confidence intervals \((\pm 90\% \text{ CI})\) and probabilistic magnitude-based inferences were reported for major outcome variables using methods outlined by Hopkins et al. (2009) (57). This approach has been used recently in similar sport nutrition and performance research (8, 96, 114, 115, 135) and provides several advantages over traditional null hypothesis testing. For example, the \(P\) value (and associated acceptance or rejection of the null hypothesis)
offers only an absolute effect or non-effect analysis within the study sample, failing to consider
the size and practical significance of this effect and whether or not it represents the true (large
sample) effect. Conversely, probabilistic magnitude-based inferences consider sampling
variability, that a range of viable values is possible over a number of small samples, together
capturing the true (large sample) effect. With only one small sample, we must provide
appropriate uncertainty as to the size of the true effect, hence the inclusion of a ±90% CI. Based
on the likelihood that the true effect falls within the CI, a qualitative inference is assigned. The
likely extent (size) of this effect is further qualified with appropriate description values.

Specifically, the smallest ‘substantial change,’ or magnitude-based effect on TT time was
calculated using a standardized coefficient for performance outcomes, 0.30, which was
multiplied by the coefficient of variation (back-transformed SD as a percent of mean) of PL (57).
For all other variables, the threshold value for the smallest substantial change was calculated
using a standardized coefficient of 0.20 (57). In both cases, inferences were assigned based on
where the CI lies in relation to the derived threshold values. For example, an effect was deemed
‘unclear’ if the ±90% CI overlapped both the upper and lower threshold value for
substantiveness. Otherwise, the likelihood of substantial treatment effect (beneficial/increase,
harmful/decrease, or trivial/negligible) was assigned using the following scale: <0.5%, most
unlikely, almost certainly not; 0.5-5%, very unlikely; 5-25%, unlikely, probably not; 25-75%,
possibly; 75-95%, likely, probably; 95-99.5%, very likely; >99.5%, most likely, almost certainly.

Confidence intervals and inferences were generated via a published spreadsheet from the
originating author (58). Specifically, the spreadsheet required the input of $P$ values, the threshold
for the smallest substantial change (described above), and the mean effect (percent difference
between treatments). $P$ values were generated via paired t-tests (2-tailed) for all variables using
SPSS® Statistics Version 21 for Windows (International Business Machines Corp.; Armonk, NY). All dependent variables (excluding certain subjective and descriptive variables) were log-transformed before analysis in order to reduce or eliminate any heteroscedasticity of residuals. Data are thus presented as back-transformed means ± SD unless otherwise specified.
CHAPTER 4

RESULTS

Subject Characteristics

Thirteen moderately trained female endurance runners and triathletes ($VO_{2peak} \geq 45.0 \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) from Florida State University and the surrounding Tallahassee area volunteered to participate in this study. All subjects were experienced with regular running training (self-reported minimum of at least 25 miles-week$^{-1}$ in the 6 months preceding the study) and competition. In certain instances, minimum running volume requirements were superseded by an adequate $VO_{2peak}$ and isovolumetric combined multiple-sport training (e.g. a triathlete who runs only 20 miles per week but also trains for swimming and cycling). All subjects reported normal menstrual physiology, six with oral contraceptive use, and six without. One subject withdrew from the study before completion due to personal circumstances, resulting in complete data from twelve subjects, the predetermined sample size (age: $30 \pm 7$ years, height: $166 \pm 5$ cm, weight: $58 \pm 4$ kg, $VO_{2peak}$: $53 \pm 4$ ml·kg$^{-1}·\text{min}^{-1}$). Inclusive anthropometric and training data are presented in Table 4.1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>29.8 ± 6.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.6 ± 4.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.2 ± 4.4</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>21.8 ± 5.1</td>
</tr>
<tr>
<td>$VO_{2peak}$ (ml·kg$^{-1}·\text{min}^{-1}$)</td>
<td>52.8 ± 4.2</td>
</tr>
<tr>
<td>Training history (yr)</td>
<td>4.3 ± 2.8</td>
</tr>
<tr>
<td>Training volume (km·wk$^{-1}$)</td>
<td>49.1 ± 18.6</td>
</tr>
<tr>
<td>Peak velocity (km·h$^{-1}$)</td>
<td>15.3 ± 1.3</td>
</tr>
</tbody>
</table>

**Note:** Data are raw means ± SD.

*N*, number of subjects; $VO_{2peak}$, peak oxygen uptake.
Dietary and Exercise Controls

Complete food and exercise log data were collected and analyzed from the study sample ($N = 12$). Subjects were instructed to record their food and exercise habits for the 72 hours preceding the first trial and replicate identically before the second trial. However, any resulting differences to diet or activity were indicated verbally to researchers and denoted in different colored ink within the logs. Subjects’ diets were analyzed for macronutrient composition (protein, carbohydrate and fat), and caffeine intake (Table 4.2). No differences to any of the aforementioned variables were observed between treatments. Of note, several subjects failed to abstain from caffeine use in the 24 hours prior to testing; however, use was consistent between trials and not present within the 6-hour window before sleep or 12-hour window before testing.

<table>
<thead>
<tr>
<th>Dietary content</th>
<th>Mean 72-hour intake</th>
<th>Mean 24-hour intake (day prior to testing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2963 ± 914</td>
<td>2863 ± 1295</td>
</tr>
<tr>
<td>CHO (%)</td>
<td>47 ± 8</td>
<td>48 ± 8</td>
</tr>
<tr>
<td>PRO (%)</td>
<td>18 ± 4</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>35 ± 10</td>
<td>33 ± 12</td>
</tr>
<tr>
<td>Caffeine (mg)</td>
<td>242 ± 284</td>
<td>278 ± 433</td>
</tr>
</tbody>
</table>

Note: Data are raw means ± SD.

$N$, number of subjects; CHO, carbohydrate; PRO, protein.

Sleep Quality and Normalcy

There were no differences (raw means ± SD) in sleep time between trials (PL: 6.7 ± 1.2 hrs, CM: 6.9 ± 1.1 hrs, $P = 0.355$). However, there was greater incidence of abnormal sleep (length and/or schedule) during CM, reported by four subjects compared to only one subject during PL. The post-prandial period was calculated from time of reported beverage consumption (nighttime) to start of exercise warm-up (morning), and was not different between trials (PL: 8.5
± 1.1 hrs, CM: 8.2 ± 1.3 hrs, \( P = 0.306 \)). There were also no differences in calculated percentage of sleep within the post-prandial period between trials (PL: 81 ± 4%, CM: 81 ± 5%).

**Appetite Assessment**

Differences (±90% CI) in 100-m VAS data between treatments are summarized in Table 4.3. Outcomes systematically reflected a decreased appetite during CM compared to PL. Respectively, CM resulted in a ‘likely small decrease’ (\( P = 0.041 \)), a ‘possibly small increase’ (\( P = 0.213 \)), and a ‘possibly trivial decrease’ (\( P = 0.289 \)) in perceived hunger, satiety and desire to eat compared to PL.

| Table 4.3 Effect of pre-sleep CM on subjective outcomes at rest (N = 12). |
|-----------------|-----------------|-----------------|-----------------|
|                 | Treatment | Outcome |                 |                 |
|                 | VAS (mm)   | PL       | CM               | Mean effect; ±90% CI (%) | Inference * |
| Hunger          | 49 ± 25    | 38 ± 20  | −22.3 ±17.4      | Small decrease likely |
| Satiety         | 41 ± 20    | 48 ± 16  | 16.9 ±23.0       | Small increase possible |
| Desire to eat   | 51 ± 27    | 46 ± 24  | −10.2 ±16.4      | Possibly trivial decrease |

*Note:* Data are raw means ± SD. Mean effect (%) represents the CM minus PL effect and the ±90% CI, the 90% confidence interval for the effect.

\( N \), number of subjects; PL, non-caloric placebo; CM, chocolate milk; VAS, 100-mm visual analogue scale.

*The inference of probability is the likelihood that the true effect of CM is substantially decreased, increased, trivial, or unclear; the threshold for the smallest substantial effect of treatment on metabolic variable is equal to 0.2 multiplied by the SD of the PL.

**Resting Metabolism**

**Energy Expenditure and Substrate Utilization.** Differences (±90% CI) in resting metabolic data between treatments are summarized in Table 4.4, along with magnitude-based inferences. Pre-sleep CM was ‘likely to increase’ RMR (\( P = 0.049 \)) compared to PL (Figure 4.1). Differences in resting VO\(_2\) closely matched differences in RMR (\( P = 0.092 \)). There were ‘unclear’ differences to resting RER between treatments (\( P = 0.848 \)).
**Blood Chemistry.** Resting blood glucose was ‘likely increased’ ($P = 0.081$) during CM compared to PL. There were no differences detected in resting blood lactate between treatments, hence the ‘unclear’ inference ($P = 0.662$). No differences to any metabolic parameters (gas exchange or blood metabolite concentrations) were related to OC use ($P > 0.05$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>PL</th>
<th>CM</th>
<th>Mean effect; ±90% CI (%)</th>
<th>Inference*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RMR (kcal·day$^{-1}$)</strong></td>
<td>1497 ± 166</td>
<td>1569 ± 172</td>
<td>4.8; ±3.7</td>
<td>Small increase likely</td>
</tr>
<tr>
<td><strong>VO$_2$ (ml·kg$^{-1}$·min$^{-1}$)</strong></td>
<td>3.7 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>4.4; ±4.3</td>
<td>Small increase likely</td>
</tr>
<tr>
<td><strong>RER</strong></td>
<td>0.78 ± 0.5</td>
<td>0.78 ± 0.4</td>
<td>0.2; ±1.7</td>
<td>Unclear</td>
</tr>
<tr>
<td><strong>Blood GLU (mg·dl$^{-1}$)</strong></td>
<td>83.7 ± 7.3</td>
<td>86.9 ± 9.0</td>
<td>3.7; ±3.7</td>
<td>Small increase likely</td>
</tr>
<tr>
<td><strong>Blood LAC (mmol·l$^{-1}$)</strong></td>
<td>0.94 ± 0.36</td>
<td>0.89 ± 0.21</td>
<td>−2.3; ±9.9</td>
<td>Unclear</td>
</tr>
</tbody>
</table>

**Note:** Data are back-transformed means ± SD. Mean effect (%) represents the CM minus PL effect and the ±90% CI, the 90% confidence interval for the effect.

*N*, number of subjects; PL, non-caloric placebo; CM, chocolate milk; RMR, resting metabolic rate; VO$_2$, oxygen uptake; RER, respiratory exchange ratio; blood GLU, blood glucose; blood LAC, blood lactate.

*The inference of probability is the likelihood that the true effect of CM is substantially decreased, increased, trivial, or unclear; the threshold for the smallest substantial effect of treatment on metabolic variable is equal to 0.2 multiplied by the SD of the PL.*

![Figure 4.1](image-url) **Figure 4.1** Effect of pre-sleep CM on morning resting metabolic rate. Data are back-transformed means. Bars are between-subject standard deviations.
Exercise Metabolism

Metabolic data from the incremental exercise portion of the protocol are summarized in Table 4.5. The resulting intensities (% VO$_{2peak}$) derived from peak velocity data were within 2.5% of the prescribed (55, 65, 75% VO$_{2peak}$). No differences to any metabolic parameters (gas exchange or blood metabolite concentrations) were related to OC use ($P > 0.05$).

Substrate Utilization. RER data are displayed in Figure 4.2a. Differences (±90% CI) to RER were observed for all three stages, each with a ‘likely’ higher value during CM versus that in PL ($P = 0.115, 0.194, 0.164$ for each progressive stage). Notably, differences were greatest at lower intensities (2.1, 1.6, 1.4% mean effect for each progressive stage).

| Table 4.5 Effect of pre-sleep CM on metabolic outcomes during incremental exercise ($N = 12$). |
|---------------------------------|---------------------------------|---------------------------------|
| Variable                        | Stage 1                         | Stage 2                         | Stage 3                         |
| Intensity (% VO$_{2peak}$)      | 54 ± 3                          | 64 ± 3                          | 74 ± 3                          |
| Treadmill velocity (km·h$^{-1}$) | 8.5 ± 0.2                       | 10.0 ± 0.3                      | 11.5 ± 0.3                      |
| RER                             |                                 |                                 |                                 |
| PL                              | 0.82 ± 0.04                      | 0.83 ± 0.03                     | 0.85 ± 0.03                     |
| CM                              | 0.84 ± 0.04$^a$                 | 0.85 ± 0.04$^a$                 | 0.87 ± 0.03$^a$                 |
| Blood GLU (mg·dl$^{-1}$)        |                                 |                                 |                                 |
| PL                              | 82.2 ± 9.2                       | 85.4 ± 8.9                      | 89.7 ± 11.2                     |
| CM                              | 81.9 ± 7.5                       | 87.1 ± 10.3$^b$                | 88.8 ± 10.5$^c$                |
| Blood LAC (mmol·l$^{-1}$)       |                                 |                                 |                                 |
| PL                              | 1.1 ± 0.4                        | 1.1 ± 0.4                       | 1.5 ± 0.6                       |
| CM                              | 1.3 ± 1.0                        | 1.2 ± 1.0                       | 1.6 ± 0.8                       |

Note: Data are back-transformed means ± SD. 
$N$, number of subjects; VO$_{2peak}$, peak oxygen uptake; RER, respiratory exchange ratio; PL, non-caloric placebo; CM, chocolate milk; blood GLU, blood glucose; blood LAC, blood lactate.

'a' Likely' higher value versus that in PL.
'b' Possibly' higher value versus that in PL.
'c' 'Likely trivial' lower value versus that in PL.

Blood Chemistry. Blood glucose data are displayed in Figure 4.2b. Blood glucose was not different between treatments during exercise at 55% VO$_{2peak}$. However, CM ‘possibly’ increased blood glucose during exercise at 65% VO$_{2peak}$ ($P = 0.358$, 2.0% mean effect), and
‘likely trivially’ decreased blood glucose at exercise intensity 75% VO$_{2\text{peak}}$ ($P = 0.561$, –1.0% mean effect). There were no systematic differences to blood lactate measurements between treatments at any incremental exercise intensity. No differences were observed for either blood glucose or lactate at the immediate- or 10-minute post 10-km TT time points (Table 4.6).

Figure 4.2 Effect of pre-sleep CM on (a) respiratory exchange ratio, and (b) blood glucose (mg·dl$^{-1}$) during incremental exercise. Data are back-transformed means. Bars are between-subject standard deviations.
Table 4.6 Effect of pre-sleep CM on post-exercise blood chemistry ($N = 12$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Immediate post-exercise</th>
<th>10-minutes post-exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood GLU (mg·dl$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>124.3 ± 27.5</td>
<td>125.1 ± 24.8</td>
</tr>
<tr>
<td>CM</td>
<td>133.6 ± 40.4</td>
<td>133.6 ± 34.3</td>
</tr>
<tr>
<td>Blood LAC (mmol·l$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>4.1 ± 6.3</td>
<td>2.5 ± 2.0</td>
</tr>
<tr>
<td>CM</td>
<td>4.7 ± 2.5</td>
<td>2.3 ± 1.4</td>
</tr>
</tbody>
</table>

**Note:** Data are back-transformed means ± SD.

$N$, number of subjects; PL, non-caloric placebo; CM, chocolate milk; blood GLU, blood glucose; blood LAC, blood lactate.

10-km Time Trial Performance

No effects of trial order ($P = 0.975$) or OC use ($P = 0.558$) were observed for TT performance. Average 10-km performance times were as follows: PL, 52.8 ± 8.4 mins, and CM, 52.8 ± 8.0 mins ($P = 0.987$, inference: ‘most likely trivial’ decrement in performance). There were no systematic differences in km-split times or treadmill speeds. However, there was a tendency for subjects to begin slower during CM, shown by a ‘possibly trivial’ reduction to 1-km speed during CM compared to PL ($P = 0.063$).

Heart Rate and Rating of Perceived Exertion

There were no systematic differences to HR at any time points (during rest, incremental exercise, or TT) between groups. Conversely, a ‘likely’ and ‘very likely’ reduction to RPE was observed at the 2- and 3-km time-points of the TT respectively ($P = 0.060$, 0.019). There were no differences between treatments at any other point.
CHAPTER 5
DISCUSSION

Several recent studies have examined the effects of acute nighttime feeding on morning metabolism and satiety in both clinical and recreationally active populations (74, 84, 133). The present investigation is the first to consider pre-sleep nutrition as a means to augment morning exercise metabolism and subsequent endurance performance in athletes, and specifically, female athletes. With nighttime CM, primary findings from the current study included next morning: 1) increased RMR, 2) increased CHO oxidation during exercise, and 3) unchanged 10-km running performance. Based on our results, we must reject our initial hypothesis that pre-sleep CM would improve next morning running performance. In addition, we may only partially accept our second hypothesis that pre-sleep CM would increase next morning CHO utilization; our RER and blood glucose data support this notion, however, our blood lactate data were unchanged and therefore do not support our original hypothesis.

Exercise Metabolism

Substrate Utilization. Our finding of ‘likely increased’ CHO utilization with CM versus PL is in agreement with previous literature that considers substrate utilization following pre-exercise CHO and/or CHO+PRO feeding compared to an overnight fast (12 hours) (2, 11, 32, 43, 100). Specifically, previous research has reported significantly increased CHO oxidation during steady-state exercise (intensity ~30-60% VO\textsubscript{2peak}) following a high-CHO pre-exercise meal compared to an overnight fast (2, 11). In the current study, RER was increased similarly during incremental exercise following CM. This pattern is likely due to a prolonged insulin response following a CHO-only or CHO+PRO meal, which is known to blunt lipolysis and consequently,
increase CHO oxidation in relation to fat (32, 91). Blood insulin typically returns to baseline values approximately 3 hours after meal consumption (32), however, this response is reported during normal, awake-hours and may differ during the nighttime feeding scenario. Likewise, in an acute study, nighttime feeding in overweight and obese women has shown increased morning insulin levels following pre-sleep feeding of meals of similar composition as the current study (40 g whey PRO, casein PRO, or CHO) (74). This outcome presents the possibility that a similar elevation of insulin levels occurred in the current study mediating the described reductions in lipolysis and increased CHO oxidation. Notably, the weight class and health status of the previously studied population may have influenced an extended response to insulin, rather than the nighttime feeding schedule. Thus, further research is required as the lack of direct blood insulin measurement presently limits insight into trends for the healthy, aerobically trained population.

Even without the blunting effects of insulin during exercise, its storage effects in the post-prandial period would allow for greater accumulation of muscle glycogen and greater availability of CHO during exercise due to a principle of ‘mass action’ (32). Additionally, it is clear that adipose tissue lipolysis increases in direct relation to the length of fasting after a meal (11), a relationship that is inversely related to CHO oxidation.

Regardless of nutritional intervention, RER will increase with increasing intensity most likely due to an increased blood catecholamine response and greater recruitment of type-II (and CHO-dependent) skeletal muscle fibers (28). The influence of pre-exercise feeding on RER seems to exacerbate this response, but does so to a greater extent during low- to moderate-exercise intensities (11). For example, the same authors that reported significantly increased CHO oxidation during post-prandial exercise $\leq \sim 60\%$ VO$_{2\text{peak}}$, showed no differences in identical
exercise completed at a higher intensity (75% \( VO_{2\text{peak}} \)) (11). In agreement, the effects on substrate utilization in the present study were also mediated by exercise intensity; the mean effect to RER between groups was greatest at lower intensities (2.1, 1.6, and 1.4% mean effect for 55, 65, and 75% \( VO_{2\text{peak}} \) respectively). In summary, RER is increased following pre-sleep CM compared to PL, which is similar to previous pre-exercise CHO interventions. However, at higher exercise intensities, workload is likely a greater determinant of substrate oxidation than pre-exercise nutrition, as reflected by a reduced effect size during later stages of progressive exercise in the current study, and previous literature (11).

**Blood Glucose.** Upon initial examination of our exercise blood glucose data, the responses between treatments would not appear to differ significantly. Values were not different between treatments during exercise at 55% \( VO_{2\text{peak}} \), with only minor fluctuations at greater exercise intensities: ‘possibly’ increased at 65% \( VO_{2\text{peak}} \) (2.0% mean effect), and ‘likely trivially’ decreased at 75% \( VO_{2\text{peak}} \) (–1.0% mean effect) following CM compared to PL. It should be noted that we did see clear differences to resting blood glucose levels between treatments, which were ‘likely’ increased following CM compared to PL. In this context, our minor changes to exercise blood glucose may be subtly suggestive of altered exercise metabolism, which is also supported by the differences observed to exercise RER.

In general, one of two outcomes is expected considering exercise blood glucose in response to pre-exercise CHO or CHO+PRO feeding versus an overnight fasting condition: 1) an augmented exercise blood glucose response (86), or 2) an early reduction in exercise blood glucose due to a *rebound hypoglycemic* effect (i.e. reduced blood glucose early in exercise following ingestion of primarily CHO due to a competing insulinemic response) (66). To the former, pre-exercise CHO would result in the additional absorption of blood glucose from the
gut to the bloodstream, increasing levels of blood glucose in circulation prior to the release of insulin (86). In this case, the rapidly absorbed blood glucose would be available as a substrate at the skeletal muscle sparing liver (and potentially muscle) glycogen, which would reflexively reduce its output (86). To the latter, *rebound hypoglycemia* occurs when the blood-glucose-lowering effects of insulin release and muscle contraction-induced GLUT-4 translocation are combined, resulting in a dramatic reduction to blood glucose early in exercise (66). The fate of the exogenous CHO is typically mediated by the timeline from CHO ingestion to the start of exercise; blood glucose is typically increased for 1-2 hours (11), and blood insulin 3 hours (32) following meal ingestion. However, again, this response is reported during normal, awake-hours and may differ during the nighttime feeding scenario.

Our data would possibly suggest greater support for augmented blood glucose during exercise following CM. Again, the fact that resting blood glucose was ‘likely’ elevated following CM would suggest an apparent pre-exercise meal effect, with or without the presence of a competing blood insulin response (not measured). The ‘possible’ increase at 65% VO$_{2\text{peak}}$ would lend further credence to this theory. On the other hand, decrements were reported to blood glucose later in exercise, albeit ‘possibly trivial.’ It is possible, but unlikely that this decrease reflects a severely blunted *rebound hypoglycemic* effect from CM. Previous research reports reductions to blood glucose occurring early in continuous exercise (15-20 mins) of similar intensity (~70% VO$_{2\text{max}}$) (32, 75). Likewise, reductions in the present study were detected following a near identical time frame, approximately 20 minutes into the exercise protocol (including warm-up and previous stages). However, as most previous research documenting this effect has utilized constant-load rather than incremental exercise, further
research is warranted to determine whether this effect was truly indicative of rebound hypoglycemia.

In total, regardless of nutritional intervention, previous research suggests an augmented blood glucose response following acute bouts of higher intensity exercise; this is due to a greater demand for energy turnover in active muscle (131), and subsequently, greater catabolism and mobilization of liver glycogen (86). Exogenous CHO, and perhaps pre-sleep CM, may exacerbate the blood glucose response, but countermands hepatic glucose output (86). Our data seemingly reflect this trend at 65% VO$_2$peak, but deviate during exercise at 75% VO$_2$peak.

**Blood Lactate.** Contrary to the previously discussed metabolic variables (RER and blood glucose), we observed no differences in resting or exercise blood lactate between treatments. It was expected that blood lactate concentrations would be increased during exercise following pre-sleep CM, as its greater accumulation would represent a greater utilization of glycolytic pathways, and thus, increased relative CHO metabolism compared to PL (21). In general, regardless of nutritional intervention, it would be expected that blood lactate would increase (similar to RER) as a result of increased workload (21). As our RER and blood glucose data generally support an increased CHO utilization following pre-sleep CM compared to PL, it is unclear why the blood lactate data fail to also demonstrate this trend.

One possible reason for this discrepancy could be the accuracy, or inaccuracy of our equipment utilized during lactate measurement. There is some evidence to suggest that our analyzer may not be as sensitive specifically for low- to moderate-levels of blood lactate (i.e. $\leq$ 4.0 mmol·l$^{-1}$ – the value most often associated with lactate threshold). For example, regardless of treatment, there was no obvious increase in blood lactate levels between stages of the IET, particularly the later stages (65-75% VO$_2$peak), which would be expected in ‘moderately-trained’
level athletes as a representation of the lactate threshold (LT), or the point when lactate production from glycolytic metabolism overcomes the rate of subsequent clearance (21). It is generally accepted that the LT exists somewhere between 50-60% VO$_2$peak in sedentary or untrained individuals (21) and may be increased to 65-80% VO$_2$peak as a consequence of endurance training adaptations (68). Based on the relative training status of our population (i.e. only ‘moderately-trained’), we would expect to see lactate data representative of the LT at an exercise intensity $\leq$ 75% VO$_2$peak (final stage of the IET). As the difference in blood lactate from resting (PL: 0.9 ± 0.2 mmol·l$^{-1}$ compared to CM: 0.9 ± 0.4 mmol·l$^{-1}$) to 75% VO$_2$peak (PL: 1.6 ± 0.8 mmol·l$^{-1}$ compared to CM: 1.5 ± 0.6 mmol·l$^{-1}$) did not encapsulate the LT (increase > 4.0 mmol·l$^{-1}$ level) it may be fair to conclude that our particular lactate analyzer was not sensitive enough to capture low-level differences to blood lactate.

Alternatively, our study sample could potentially have an average LT slightly higher than 75% VO$_2$peak, as there were clear increases to blood lactate above resting values immediately post-TT (PL: 4.1 ± 6.3 mmol·l$^{-1}$ compared to CM: 4.7 ± 2.5 mmol·l$^{-1}$). While no VO$_2$ data were collected during the TT, which would serve as an exact index to compare intensity, it is interesting to note that the average treadmill speed was slightly higher during the last km of the TT (PL: 12.3 ± 1.6 km·h$^{-1}$; CM: 12.2 ± 1.7 km·h$^{-1}$) than during the 75% VO$_2$peak stage of the IET (11.5 ± 1.0 km·h$^{-1}$), a trend that is similar considering our heart rate data. However, we still might expect that lactate data during such a high-intensity effort as the 10-km to be substantially higher even than the LT.

**Effect of the Post-Prandial Period.** In general, the effects observed to exercise RER and blood glucose in the current intervention support the findings of previous research that considers exercise metabolism following consumption of comparable CHO or CHO+PRO
nutrition, however, notably, the effects in our intervention are of comparatively smaller magnitude. One potential explanation for this discrepancy is perhaps the same factor that makes the findings of the current study particularly novel: the duration of the fasting period after which effects were still observed. Previous research by Montain et al. (1991) has determined an upper limit of 4 hours for a post-prandial period in which effects on exercise metabolism were still present (91). Our data show similar effects to exercise metabolism (i.e. increased RER) that extend nearly 8.5 hours out from meal consumption, markedly longer than the previous 4-hour threshold. Importantly, this may be due to our use of more refined statistical analysis, allowing for greater sensitivity in detecting small but meaningful outcomes.

Our mean effects were blunted compared to the mean effects reported from Montain’s group (e.g. a 1.6-1.4% increase in RER versus a 13.0% increase in CHO oxidation in g·min\(^{-1}\) during exercise at comparable exercise intensity, respectively). A trend that is similar for blood glucose data (75). In regards to Montain’s findings, this difference of magnitude is likely due to the substantially greater caloric bolus of the pre-race meal (which was over three times that of the present treatment: approximately 605 kcal based on information given compared to 180 kcal from CM in the present study) (91). The greater caloric density, and specifically, greater CHO density (132 g CHO compared to 30 g CHO) of a pre-exercise meal has been shown previously to augment the subsequent exercise metabolic response (119). In addition, the shorter duration of the post-prandial period would provide less time for CHO storage after digestion and absorption from the gut, increasing the availability of metabolites in the blood stream (63), which would reflect a greater blood glucose response. Both facts likely contributed to the more substantial effects reported by Montain et al. (1991) and the like (2, 32) compared to the present data.
**Effect of Post-Prandial Sleep.** It is very difficult to compare the current literature to previous findings. Besides differences likely mediated by timing and meal size, our intervention introduces one novel element: sleep imposed within the post-prandial period. Our findings suggest that sleep may increase the duration of post-prandial effects, possibly due to slowed digestion.

While the specifics of digestion and absorption kinetics during sleep are largely unknown, it appears that gastric motility is reduced (46, 73). The potentially slowing of digestion during sleep may be partially explained by the effects of body position on digestion and absorption kinetics. It has been reported that the digestion of whole-food (standardized breakfast: 528 kcal; 70 g CHO; 19 g PRO; 20 g FAT) and liquid meals (commercially available milk: 134 kcal; 15.3 g CHO; 7 g PRO; 3 g FAT) of primarily carbohydrate is extended when the post-prandial period includes resting in the supine position compared to sitting (55, 56). The slowed gastric emptying in the supine position allows for greater overall absorption of CHO from the gut to the bloodstream (55, 56). This is likely facilitated by autonomic control, specifically, increased parasympathetic nervous system activity in the supine position compared to seated (21, 55, 56), which to a large extent modulates the enteric nervous system in the gut (73), hence the associated phrase: ‘rest and digest.’ There is little information considering the enteric nervous system exclusively in regards to posture or sleep, but as it is known that the relative contribution of the parasympathetic nervous system branch is generally increased during sleep in the supine position (94), it is reasonable to speculate that enteric activity may also be enhanced, allowing for the greater absorption of pre-sleep CHO (CM in this case), and the extension of the period of absorption from gut to bloodstream.
Furthermore, the previously mentioned research utilized a milk-based beverage, a near direct comparison to CM. CM contains galactose as a primary CHO (63) and casein and whey PRO at a 3:1 ratio (125); both characteristics are known to independently require longer digestion and absorption compared to other forms of CHO and PRO respectively (63, 125). The unique composition in combination with the liquid nature (small volume) of CM may have further reduced the rate of gastric emptying compared to an alternative whole-food meal option (35). CM may therefore be an ‘ideal’ candidate for pre-sleep nutrition, as it’s characteristics may independently extend the period of digestion and absorption that is already prolonged due to body position (55, 56) and/or sleep (46, 73). Finally, the moderately-trained status of our study population likely exacerbated digestion efficacy, as parasympathetic nervous system activity is known to be higher in trained adults compared to untrained over a 24-hour period, including sleep (48).

This culmination of evidence may explain the apparent withstanding effects to resting and exercise blood chemistry and RER in the morning following PL compared to CM; that is, the lengthened state of absorption resulted in a subsequent extension in the typical blood glucose elevation following a meal (typically 1-2 hours (66)) to nearly 8.5 hours in the present study, with effects subsequently translated to exercise (typically 4 hours (91)). Similarly, both Res et al. (2012), and Groen et al. (2012) have demonstrated effective absorption of casein PRO in the overnight period, with plasma amino acid concentrations elevated for nearly 7.5 and 5 hours post-prandial respectively (49, 107), lending credence to this theory. In total, the gut appears to effectively digest and absorb CHO and PRO during sleep, with extended effects to support the metabolism of the morning endurance athlete.
While not directly assessed by the current intervention, it is interesting to note the relative contribution of sleep- and awake-hours that comprised the post-prandial period. Following both treatments, it was estimated that sleep could account for approximately 81% of the post-prandial period. If sleep were, in fact, the true mediator in extending metabolic effects, it would be important to determine the appropriate timeline and threshold to maximize effects. Future research should consider nighttime feeding using a systematic method to assess dose-response.

**Sleep Quality and Normalcy**

Importantly, whether or not related to the CM supplement, there was a higher incidence of atypical sleep reported during the CM trial versus that in PL. However, subjects reported reduced length of sleep, in most cases due to a later bedtime, rather than a sleep disturbance occurring during the night or early morning. In previous research with casein PRO, whey PRO, or CHO, no adverse effects had been reported in terms of sleep quality, distress, or nausea (49, 74, 84, 107).

However, and perhaps vindicating the age-old claim, there is some evidence to suggest that the acute pre-sleep consumption (20) and habitual dietary intake (136) of milk-based proteins may actually improve subsequent sleep length, quality, and onset. Importantly, the population and exact nature of milk-based products studied previously varies slightly from that of CM. We may only speculate about these claims, as the absence of direct measurement in the present study limits the conclusive evidence of CM in regards to subsequent sleep typicality or atypicality.
Appetite and Resting Energy Expenditure

Our finding of systematically reduced appetite following CM compared to PL is in agreement with previous nighttime feeding research, most notably findings from Groen et al. (2012), Kinsey et al. (2014), and Ormsbee et al. (2014). The previous literature has reported subjective appetite as a potential strategy for long-term weight management or weight gain (a benefit for the elderly population). In the case of the morning athlete, our primary finding of reduced hunger (‘likely small decrease,’ $P = 0.041$), is most likely related to the satiating effects of PRO in CM (19, 78). In relation to performance, reduced hunger before exercise is likely advantageous to the comfort and perceived “race-readiness” of the athlete, particularly in the absence of during-exercise feeding, such as the present study.

Our findings of enhanced RMR following CM compared to PL are in agreement with previous nighttime feeding research (74, 84). Clearly, the consumption of additional calories, regardless of macronutrient profile, will increase thermogenesis, and subsequently total energy expenditure compared the consumption of nothing (i.e. PL). It was hypothesized previously that nighttime feeding of specifically PRO would increase RMR to a greater extent than CHO (due to reported enhanced thermogenic effects following PRO (67, 93)), however this effect was not observed (74, 84). As CM contains both CHO and PRO, our intervention offers no further clarification on the effects of singular macronutrients on RMR in the nighttime feeding scenario.

10-km Time Trial Performance

Despite the noted enhancements to morning metabolism, performance was unaltered (i.e. ‘most likely trivial’ differences). Other research has mixed results considering pre-exercise CHO+PRO and subsequent performance (3, 14, 59), however to our knowledge, no previous research has utilized a 10-km TT with positive results. It is possible that this particular
performance protocol is not sensitive enough to determine effects of nutritional interventions, however it is unquestionably specific to the high-intensity, relatively short-duration athlete in question. Our study sample included moderately trained women, who at times were multi-sport athletes, rather than exclusively runners. This difference in training background and racing emphasis could have likely lent some variability to our results. Performance differences may have been more apparent with more ‘elite’ level female athletes whose training was exclusive to running. Also, there is the difficulty of self-paced treadmill running, which may have been less than truly reflective of performance. Of note, there was a tendency for subjects to ‘pick a speed’ and try to maintain it, rather than run more variably as they likely would during a real competition. This could have further limited findings. In addition, there was high variability in some instances both between trials and between previously reported best 10-km times – in large enough magnitude that it was unlikely due to the intervention, and more likely due to lack of consistent pacing ability on the treadmill. For example, on average, the subjects reported a previous best of 47.0 ± 6.1 mins, nearly 6 mins faster than their performance in the current (PL: 52.8 ± 8.4 mins compared to CM: 52.8 ± 8.0 mins). Of course, the described limitations are apparent in all literature utilizing a TT approach for performance testing, but may be particularly noteworthy in the current intervention. Future studies might consider alterations to the traditional TT approach or more stringent familiarization.

Besides lack of difference to finishing time, there were some distinct patterns to pacing profile and perceived effort between treatments. In particular, subjects who ingested CM began the TT at a ‘likely trivial’ reduced treadmill speed (1-km) compared to PL trial, with no, or a ‘most likely trivial’ decrement to finish time. There is some evidence to suggest that for long-distance track events such as the 5-km and 10-km distances that a ‘negative’ or ‘even’ split
pacing strategy is most advantageous to performance (45, 128). As both strategies were present in the current study and resulted in ‘most likely negligible’ differences to performance, it is unclear whether one pacing profile was more advantageous than the other.

On the other hand, one factor that might argue the case for CM was a reduced perception of exertion early in the TT. Specifically, RPE was ‘likely’ and ‘very likely’ lowered at the 2- and 3-km points of the TT comparatively. One potential explanation for this was the above-mentioned decreased start speed during CM compared to PL. However, this inference was ‘likely trivial’ and may therefore not fully explain the reduced RPE. Additionally, it should be noted that there was no compensatory increase in RPE later in exercise (as intensity presumably increased), meaning that overall, total perceived effort was reduced during CM compared to PL. The relevance of this effect is questionable considering that there was no physiological explanation for a decrease in RPE (e.g. reduced heart rate) and performance was unaffected.

Limitations

One major limitation in the current intervention was the inability to monitor subjects overnight. The absence of blood chemistry data for this time period or indices of sleep quality makes it more difficult to interpret the physiological and practical implications of pre-sleep nutrition. Therefore, it may only be speculated as to how nighttime digestion and absorption kinetics may have extended metabolic affects, and whether or not this disrupted normal sleep patterns. Secondly, blood insulin data may have provided additional information about the morning regulatory state (i.e. relative storage versus mobilization) of subjects, and thus further clarity of the timeline of events from pre-sleep meal to morning measurement.

A final limitation is the lack of direct comparison to an identical morning pre-exercise meal. This would allow us to better quantify differences in effect size (if any) based on the
extended time period between a morning treatment and pre-sleep treatment. The exact same size and content of treatment would make the current comparison more robust, and should be considered for future research.

Conclusions

In summary, a pre-sleep meal of CM may enhance next morning resting and submaximal exercise metabolism, most notably by increasing CHO utilization, although this was not supported by blood lactate data. This is the first research to show effects to exercise metabolism greater than 4 hours after meal ingestion. It is speculated that dormancy within the post-prandial period mediated effects via unique digestion and absorption kinetics, which were possibly extended. The exact threshold for effects based on the proportion of sleep within the post-prandial period remains to be elucidated; however an approximate 81% fraction (sleep hours/post-prandial hours) appears sufficient. While the effects observed to metabolism are novel, they did not translate to improved running performance. However, there was a tendency for subjects to alter their pacing strategy to negative split, and for reduced perceived effort early in exercise. Future research might consider different exercise modalities or protocols to more sensitively assess performance.
APPENDIX A

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 obtainment of a relative maximal oxygen consumption ($V_{O2max}$) value greater than or equal to 40.0 ml/kg/min. $V_{O2max}$ represents the maximal ability of the body to utilize oxygen and is representative of cardiorespiratory fitness.

3. My participation in this study will require coming to the Human Performance Laboratory at The Florida State University for a $V_{O2max}$ 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)³. 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).

Initials

I will then complete a VO\textsubscript{2max} test using a graded treadmill (Woodway PPS Med; Waukesha, WI) and metabolic cart system (Parvo Medics Truemax 2400 Metabolic Measurement System; Consentius 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\textsuperscript{TM} 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 VO\textsubscript{2max} 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

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-10S).

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$_{2\text{max}}$ determined from VO$_{2\text{max}}$ 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
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 \( \text{VO}_{2\text{max}} \) 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 investigatory, 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 [redacted] Katie Gorman at [redacted]
or Beth Miller at [redacted] for answers to questions

Initials

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 [redacted].

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 B
IRB APPROVAL MEMORANDUM

Office of the Vice President For Research
Human Subjects Committee
P O Box 3062742
Tallahassee, Florida 32306-2742
(850) 644-8673 · FAX (850) 644-4392

APPROVAL MEMORANDUM (for change in research protocol)

Date: 09/05/2014
To: Michael Ormsbee <mormsbee@fsu.edu>
Address: 1493
Dept: NUTRITION FOOD AND EXERCISE SCIENCES
From: Thomas L. Jacobson, Chair
Re: Use of Human subjects in Research
Project entitled: Effect of Nighttime Feeding on Morning Performance in Female Endurance Athletes

The application that you submitted to this office in regard to the requested change/amendment to your research protocol for the above-referenced project has been reviewed and approved.

Please be reminded that if the project has not been completed by 04/08/2015, you must request renewed approval for continuation of the project.

By copy of this memorandum, the chairman 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 IRB00000446.

Cc: Robert Moffatt <rmoffatt@fsu.edu>, Chair
HSC NO. 2014.13087
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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The training and education completed in the protection of human subjects or human subjects records:

FSU Training Module   NIH   HIPAA

Occupational Position: Faculty

1.3 Co-Investigators/Research Staff

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FSU Training Module

Occupational Position: Student

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APPENDIX C

HEALTH HISTORY QUESTIONNAIRE

Date: _________________________________                        ID#: ___________

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: ___________________________________________________ ____________________
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

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<th>Disease/Operation</th>
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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:

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?

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

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 ______

_______________________________           __________________
Subject Signature                                            Date
# 2014 Calendar

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APPENDIX D

ENDURANCE TRAINING QUESTIONNAIRE

Date: _________________________________              ID#: ________

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:

__________________________________________  ____________________________

Subject Signature                                  Date
APPENDIX E

3-DAY FOOD AND EXERCISE LOG

Date: _________________________________                       ID#: _________

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

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

Source: © 2015 CSL Nutritional Services (77).
**SAMPLE**

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<th>Serving size</th>
<th>Food item</th>
<th>Specific activity</th>
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<tr>
<td>7:30 am</td>
<td>1 cup</td>
<td>Cheerios</td>
<td>Sat on the couch</td>
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<td></td>
<td>½ cup</td>
<td>2% milk</td>
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<tr>
<td></td>
<td>1 cup</td>
<td>Apple juice</td>
<td></td>
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<td>8:30 am</td>
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<td></td>
<td>Recovery run (4 miles around 8:30 pace)</td>
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<tr>
<td>10:00 am</td>
<td>1 medium</td>
<td>Banana</td>
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<td></td>
<td>1 cup</td>
<td>Water</td>
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<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>
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<tr>
<td></td>
<td>1 slice</td>
<td>Cheddar cheese</td>
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<td>1 patty</td>
<td>Hamburger</td>
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<td>1 supersized</td>
<td>French fries</td>
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<td>1 16 ounce</td>
<td>Regular coke</td>
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<td>3:30 pm</td>
<td>15</td>
<td>Crackers - Sociables</td>
<td>Worked at desk (seated)</td>
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<td></td>
<td>2 Tbsp</td>
<td>Peanut butter</td>
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<td>1; 8 ounce</td>
<td>Juice box</td>
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<td>6:30 pm</td>
<td>5 ounces</td>
<td>Chicken -thigh - baked</td>
<td>Watched TV</td>
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<td>1 ½ cups</td>
<td>Rice</td>
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<td>½ cup</td>
<td>Broccoli</td>
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<td>1 cup</td>
<td>2% milk</td>
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<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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<td></td>
<td>3 Tbsp</td>
<td>Chocolate sauce</td>
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<td>8:30 pm</td>
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<td>Did house chores</td>
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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

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

Was this a fairly typical day for you in terms of food intake and exercise? Explain
** Do not forget to record beverages, including water

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


38. Emanuele M, Wezeman F, Emanuele N. Alcohol’s Effects on Female Reproductive Function (Figure 1). *Alcohol Res Heal.* 26: 274–81, 2002.


86. **Marmy-Conus N, Fabris S, Proietto J, Hargreaves M.** Preexercise glucose ingestion and glucose kinetics during exercise. 1996.


BIOGRAPHICAL SKETCH

Katherine (Katie) Gorman is originally from the greater Philadelphia area and received her Bachelor’s of Science degree in Kinesiology from James Madison University in Harrisonburg, Virginia (Spring 2013). While at James Madison, Katie was a scholarship athlete and competed in Cross Country and Track and Field under Coach Dave Rinker. She became interested in Exercise Physiology primarily due to the exceptional energy, commitment, and standards of her undergraduate professors in the Kinesiology department, most notably Dr. Christopher Womack and Dr. Nicholas Luden. During her junior year, Katie was honored to receive a departmental scholarship – the Bruce Crawford Morrison Rummel Scholarship, awarded for the promotion of girls and women in sport and exercise, a cause that would be a driving force for her future research. During her final semester, she also received the Outstanding Major in Exercise Science award from her department.

In Fall 2013, Katie continued her studies to the graduate level at Florida State University in Tallahassee, Florida. She is currently a Master’s of Science degree candidate in Exercise Physiology under the direction of Dr. Michael J. Ormsbee. During her master’s work, she has had the opportunity to work on several research projects, including that of her peers and most notably, the current thesis. Katie is thrilled to possibly contribute to the enhanced understanding of the female athletic population, and hopes to build upon this small impact in her future research. Katie presented her work at the 2015 Southeast American College of Sports Medicine Annual Conference. She plans to defend her thesis and graduate from the College of Human Sciences in May. In her spare time, Katie enjoys continued training and competing in endurance athletics, following Women’s Track and Field, oil painting, eating great food, and spending time with her friends and family.