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Melissa Eryn Stack

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THE EFFECTS OF CHRONIC L-CARNITINE AND CARBOHYDRATE
SUPPLEMENTATION ON BODY COMPOSITION AND ATHLETIC PERFORMANCE IN
FEMALE ENDURANCE ATHLETES

by

Melissa Stack

(Under the Direction of Amy-Jo Riggs)

ABSTRACT

Purpose. The purpose of this study was to examine the effects of chronic L-carnitine and carbohydrate supplementation on body composition, perceived exertion, and athletic performance in recreationally-trained female endurance runners. **Methods.** On three separate occasions, twenty-one days apart, seven recreationally-trained female endurance runners performed a timed progressive treadmill test to exhaustion as well as a body composition assessment using the Bod Pod®. Participants were randomly assigned to one of two groups – L-carnitine (LC) or L-carnitine plus carbohydrates (LC + CHO). The LC group consumed 2 g of L-carnitine L-tartrate and the LC + CHO group consumed 2 g of L-carnitine L-tartrate with 10 g of flavorless dextrose powder. Rating of perceived exertion, time to fatigue, and body composition was recorded during each of the three visits. **Results.** The results of this study revealed no significant interactions or main effects for time and treatment ($p = .11$). In addition, no significant differences were seen between subjects for body composition (%FFM $p = .54$; FFM $p = .17$; FM $p = .75$; %FM $p = .57$) or RPE ($p = .95$). **Conclusion.** In conclusion, this study demonstrated that chronic carnitine and carbohydrate feeding likely does not influence RPE, time to fatigue, or body composition in recreational endurance-trained female runners.

INDEX WORDS: L-carnitine, Carbohydrates, Rating of perceived exertion

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by

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B.S., Georgia Southern University, 2015

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A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial

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MASTER OF SCIENCE

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CHAPTER 1

INTRODUCTION

Carnitine, a nonprotein amino acid, is found naturally in the body, mainly in the muscles, and is synthesized from the amino acids lysine and methionine (Orer & Guzel, 2014). Not only is it found naturally in the body, but L-Carnitine (LC) can be found in numerous dietary food sources including red meats and dairy, and in smaller amounts in fish, poultry, asparagus, tempeh, and peanut butter. Carnitine can also be found as a supplement in various forms, including Acetyl L-Carnitine, Propionyl-L-Carnitine, and the most widely available, L-Carnitine (Carnitine Shuttle; Ehrlich, 2014). Carnitine is necessary for the important role of shuttling fatty acids, specifically long-chain fatty acids, across the inner mitochondrial membrane for beta-oxidation (Karahan, Coksevim & Artis, 2010). This transport system is the critical rate-limiting step for beta-oxidation. Thus, if this transport system is continuously in motion, beta-oxidation will occur at a normal rate, allowing the fatty acid molecules to be broken down in the mitochondria to generate acetyl-CoA, and eventually ATP.

Carnitine supplementation has become increasingly popular over the years, claiming to promote fat loss, increase energy, and help prevent an increase in body fat mass. However, the benefit of carnitine supplementation in individuals without deficiency is widely up for debate (Watcher et al., 2002). Until recently, few researchers have found significant evidence that substantiates carnitine feeding to enhance athletic performance. Nevertheless, research now supports evidence that carnitine supplementation, in combination with carbohydrates (CHO), can produce ergogenic effects in individuals without deficiency (Broad et al., 2011; Stephens et al., 2013; Wall et al., 2011).

As previously mentioned, one of the major claims associated with L-carnitine supplementation is its potential “fat burning” capability, however, research associated with carnitine supplementation and fat loss is limited and some studies do not support the use of LC for fat loss purposes. For instance, Kruszewski (2011) examined changes in maximal strength and body composition after the ingestion of creatine, L-carnitine, or β -hydroxy- β -methylbutyrate (HMB) among novice male body builders with little to no training experience. Participants in the carnitine group consumed either an L-carnitine supplement or placebo, both at 900 mg per day for a total of 1.2 months. Over that time period, participants were instructed to perform circuit training-like exercise three times a week at the same time each day. Bioelectrical impedance analysis (BIA) was then used to determine fat and water content in the body. Results showed no significant changes in fat or water content in the LC group, as well as no significant changes in body mass. These findings do not support the claims currently made by supplement companies, in that carnitine supplementation did not have a significant effect on body composition in these subjects.

Similarly, Wutzke and Lornez (2004) examined the effect of L-carnitine on fat oxidation, protein turnover, and body composition in twelve slightly overweight participants (7 females, 5 males; age 18 to 30 years). Participants in the study had kept their weight stable for at least one month prior to the start of the study. For twenty days, participants were provided with a regular, individualized diet either with or without 3 g of carnitine per day (10 days with LC, 10 days without). In order to assess protein turnover, an algae lipid mixture was given to participants simultaneously on the ninth day. Urine samples were collected at different intervals over a period of 36 hours after administration of the mixture. In addition, BIA was used to determine body fat mass (BFM), total body water (TBW), lean body mass (LBM), and body weight (BW). In this

population, results showed no change in BFM (21.3 ± 4.8 vs 21.5 ± 5.1 kg), LBM (58.1 ± 10.8 vs 58.3 ± 10.6 kg) TBW (42.5 ± 7.9 vs 42.7 ± 7.8 L), and BW (79.4 ± 10.5 vs 79.7 ± 11.5 kg) at the end of the supplementation period. Once more, few studies have been able to support the use of carnitine supplementation in preventing body fat accumulation, therefore more research is necessary to substantiate this claim.

Other interests in carnitine spur from the claim that supplementation has the potential to improve athletic performance due to carnitine's role in energy metabolism. Through the carnitine transport system, fatty acids are able to enter the mitochondria to be broken down for subsequent B-oxidation, specifically during endurance exercise, where the reliance of fatty acids as a fuel source becomes higher. Watcher, Vogt, & Kreis (2002) investigated the effects of long-term L-carnitine supplementation on skeletal muscle carnitine content and physical performance in eight healthy male adults. Using a cycle ergometer, participants exercised for 10 minutes at 20%, 40%, and 60% of their individual maximal workload (P_{max}) until exhaustion. Afterwards, participants were instructed to consume 2 g of LC two times per day for 3 months. Exercise tests and muscle biopsies were performed before, immediately after, and two months after the treatment. Results of the study revealed no significant changes in VO_2 , RER, blood lactate concentrations, or heart rate when compared to pretreatment values after 3 months of LC supplementation. In addition, a 5% increase was seen in VO_2 at submaximal workloads (40% and 60% of P_{max}) when compared to pretreatment values, however, VO_{2max} , HR, VO_2 at 20% P_{max} , RER, and blood lactate concentrations were not significantly different from values obtained before the treatment.

Furthermore, recent research has reported that ingestion of L-carnitine in combination with carbohydrates has been shown to prolong exercise endurance as well as aid in the recovery of muscles post-exercise. Wall, Stephens, & Constantin-Teodosiu (2011) examined muscle

carnitine content and alterations in muscle fuel metabolism during exercise in fourteen moderately trained athletes. On three separate occasions, participants reported to the lab to complete a 60-minute cycling test for 30 minutes at 50%, followed immediately by 30 minutes at 80% of their predetermined VO_{2max} . Rating of perceived exertion (RPE) was recorded every ten minutes. After the first visit, participants were separated into either a control (80 g orange-flavored CHO polymer) or carnitine group (80 g orange-flavored CHO polymer plus 1.36 g of LC). Blood samples and muscle biopsies were taken on each visit to examine plasma total carnitine (TC) concentration and muscle free carnitine, acetylcarnitine, and long-chain acylcarnitine. After 24 weeks of supplementation, results of the study revealed higher plasma TC concentrations after 12 and 24 weeks in the carnitine group when compared with control, and a 35% decrease in muscle glycogen content after exercise at 50% VO_{2max} . In addition, after exercise of 80% VO_{2max} , a 44% decrease was seen in muscle lactate content in the carnitine group compared to control. Work output also increased by 11% from baseline after 24 weeks, 35% greater compared to control. These findings are the first to show that carnitine concentrations in the muscle can be increased through dietary means and may be exercise-intensity dependent. Wall and colleagues (2011) also demonstrated that at lower intensities (50% VO_{2max}), increasing muscle carnitine content spares muscle glycogen, thus utilizing lipids as a fuel source.

Various limitations and inconsistencies exist in current literature involving carnitine supplementation. Research involving carnitine supplementation has produced conflicting results, probably due to small sample sizes, inconsistent duration of supplementation, and the use of mainly male participants. For example, Broad et al. (2011) examined L-Carnitine L-Tartrate (LCLT) supplementation on sixteen endurance-trained male athletes over a 15-day period and

found significant differences in mean exercise heart rate (HR) and rating of perceived exertion when participants performed an exercise test on a cycle ergometer at varying intensities.

Similarly, Mustafa, Beker, & Artis (2010) found that ten days of carnitine supplementation in twenty male, well-trained athletes produced significant changes in 1500m running performance time and plasma lactate levels in their control group (fruit juice) compared with the carnitine group (2 g/day LCLT mixed with fruit juice). In addition, Swart, Rossouw, & Loots (1997) found significant differences in running speed (5.68% increase from baseline), heart rate, and respiratory exchange ratio when examining the effect of L-carnitine supplementation on plasma carnitine levels and different performance parameters in seven male marathon athletes over a period of six weeks. Participants performed two progressive treadmill tests to exhaustion, and results of the study showed a 5.68% increase in running speed, as well as a decrease in oxygen consumption and heart rate when compared to baseline.

In addition to inconsistent sample size and length of supplementation, most studies use predominantly male or mixed gender samples, whereas few studies have used exclusively female samples. Within the last few decades, important differences in exercise capacity between males and females has been brought to light (Sheel, Richards, Foster, & Guenette, 2004). For instance, compared to males, females tend to utilize more lipid and less carbohydrate for energy during endurance exercise. In addition, fatigability of skeletal muscle appears to be different when comparing genders, which could be related to differences in muscle mass, substrate utilization, and recovery (Sheel, Richards, Foster, & Guenette, 2004). Howden, Perhonen, & Peshock (2015) examined the effect of cardiovascular response to one year of intensive endurance training on previously untrained males and females, matched for training volume and intensity. Each participant underwent various tests every three months to assess body composition as well as

VO_{2max} , left ventricle mass (LV), and cardiac magnetic resonance imaging (MRI) testing. Results of the study found that compared to males, female participants had a lower percentage of fat free mass (FFM) and lower hematocrit levels before training. However, upon completion of the study protocol, there were no significant differences between sexes for hematocrit, body fat, and indexed blood volume. Furthermore, VO_{2max} was increased in both sexes (22% in males, 15% in females), but VO_{2max} response was blunted after three months in female participants compared to males. In the female population, VO_{2max} , LV mass, and mean wall thickness plateaued after 3 months of training, whereas those same measures increased in male participants before plateauing in months 9-12. As mentioned in the study, one reason for the plateau in exercise performance in the female population may be related to the groups suboptimal energy intake, as the study protocol only provided general dietary advice (Howden, Perhonen, & Peshock, 2015). This study is one example of the different physiological responses to exercise between males and females. Other factors that may have contributed to the lack of training effect in exercise performance include hormonal and environmental factors (Howden, Perhonen, & Peshock, 2015).

Likewise, an important factor to consider when working with the female population and exercise is the use of oral contraceptives. Joyce, Sabapathy, Bulmer, & Minahan (2013) examined the effect of long-term oral contraceptive use on the endurance performance of recreationally active women. Plasma ethanol estradiol, a hormone found in many oral contraceptives, inhibits the production of the hormone 17 B-estradiol, and appears six times higher in individuals taking oral contraceptives than those that do not. For this study, participants were separated into two groups: oral contraceptive (OC) and those who were not using oral contraceptives (CON). Baseline samples were then taken to assess levels of 17 B-estradiol and

progesterone. VO_{2peak} and anaerobic threshold (AT) were measured using a cycle ergometer where participants completed various exercise tests to exhaustion. Heart rate (HR), RPE, pulmonary gas exchange, blood lactate concentration (La) and time to exhaustion were assessed during the tests. Significant differences were observed in VO_{2peak} and VO_2 at AT in the CON group when compared to the OC group. However, HR, BP, and La concentrations were not significantly different between the groups. Results of this study propose that long-term use of oral contraceptives negatively affect VO_{2peak} and VO_2 at AT, but have no effect on athletic performance in recreationally trained female endurance runners.

Overall, the importance of the current study lies in the fact that few researchers have been able to produce consistent results with LC supplementation and studies involving carnitine and CHO supplementation are relatively novel. The current study set out to examine the effect of chronic L-carnitine and carbohydrate feeding on overall on body composition and athletic performance in female recreational endurance runners. Chronic carnitine and CHO supplementation (> 4 weeks) has given promising insight into changes in fuel metabolism and recovery after exercise. It was hypothesized that participants receiving the CHO + LC would have a significantly higher work output and would have lower feelings of fatigue when compared to LC group. In addition, it was hypothesized that participants receiving the CHO + carnitine supplement will see no increase in fat mass when compared to LC group.

Purpose Statement:

The purpose of this study was to examine the effects of chronic L-carnitine and carbohydrate supplementation on body composition, perceived exertion, and athletic performance in female endurance athletes.

Hypotheses:

1. Subjects receiving the CHO + carnitine supplement will have a significantly higher work output when compared to the LC group.
2. Subjects receiving the CHO + carnitine supplement will have lower feelings of fatigue when compared to LC group.
3. Subjects receiving the CHO + carnitine supplement will see no increase in fat mass when compared to LC group.

Research Questions:

1. Does chronic carnitine and CHO feeding increase work output?
2. Does chronic carnitine and CHO feeding reduce the feeling of fatigue?
3. Does chronic carnitine and CHO feeding prevent increases in body fat mass?

Rationale:

Previous research has produced conflicting results when it comes to L-carnitine (LC) supplementation and its effect on athletic performance. The importance of this study lies in the fact that few researchers have been able to produce consistent results with LC supplementation and, furthermore, studies involving LC and carbohydrate (CHO) supplementation are relatively modern. Thus far, chronic LC and CHO supplementation (> 4 weeks) has given a promising insight into enhancing athletic performance by reducing the physiological effects of exercise and improving recovery. Currently, many studies have been unable to support the use of carnitine

supplementation to enhance athletic performance, however these conclusions have been drawn based on small numbers of participants, short duration of supplementation, lack of appropriate control groups (Higdon, 2002), type of supplementation, or control of diet before exercise (Abramowicz & Galloway, 2005), so further research is necessary in this area. Therefore, the purpose of this study was to examine the effect of chronic L-carnitine and carbohydrate feeding on athletic performance, body composition, and perceived exertion in female endurance athletes.

Limitations:

- Random assignment of supplement groups (carbohydrate + L-carnitine or L-carnitine)
- Inability to collect blood samples for analysis
- Small sample size
- Not regulating menstrual cycle
- Spring Break fell in the middle of the study protocol, postponing testing for some of the participants.
- Other limitations to this study included possible threats to internal and external validity including history of gastrointestinal or metabolic disorders, mortality, maturation, testing and instrumentation, as well as the reactive or interaction effect of testing.

Delimitations:

Participants for this study consisted of recreationally active females, ages 19-22 years, who were participating in at least 150 minutes of endurance exercise a week. Participants were familiar with the addition of CHO supplements in their dietary regimen and were not taking any performance enhancing drugs or any medications that may affect body composition or appetite. Participants ceased any additional supplementation regimen for the duration of the study and

maintained their training regimen and diet. Participants also must have been taking oral contraceptives for at least six months prior to the first trial.

Assumptions:

1. Participants will return for all required exercise protocols.
2. Participants will take their required dosages of the supplement they are given.
3. Participants will take their required supplements at the correct time and frequency throughout the study.
4. Participants will not be taking any performance enhancing drugs at the time of the study
5. Participants will continue with their current diet and training regimen throughout the duration of the study
6. The Bod Pod® is a reliable and valid measure of body composition
7. The ParvoMedics TrueOne® 2400 proves to be an accurate and reliable device for the measurement of gas exchange variables

Operational Definitions:

1. Exhaustion: A state of extreme physical or mental fatigue
2. Carnitine: An amino acid derived from the amino acids, lysine and methionine. Carnitine plays a crucial role in the transport of fatty acids into the mitochondria.
3. VO_{2peak} : Also known as maximal oxygen uptake; refers to the maximum amount of oxygen that an individual can utilize during intense or maximal exercise. VO_{2max} is one of many factors that can help predict an athlete's ability to perform sustained exercise.

CHAPTER 2

LITERATURE REVIEW

Carnitine in the Human Body

L-Carnitine is just one of the many critical components involved in the metabolic process of beta-oxidation. The most widely researched aspect, and primary function, of this amino acid-like substance is its role in the transportation of long-chain fatty acids (LCFAs) across the inner mitochondrial membrane (Karlic & Lohninger, 2004) for use as fuel by the body. With the main sites for carnitine storage being cardiac and skeletal muscle (Higdon, 2002), carnitine also acts as a buffer for excess acetyl-CoA in the muscle that accumulates during exercise (Broad, Maughan & Galloway, 2005). This buffering mechanism is thought to relieve the inhibition of the pyruvate dehydrogenase enzyme complex (PDC), which in turn increases glycolytic flux during exercise (Broad et al., 2005). The main sites for carnitine synthesis include the liver and kidney, where carnitine can then be transported to other tissues in the body.

Two main enzymes - carnitine palmitoyltransferase I (CPT I) and carnitine palmitoyltransferase II (CPT II) - make up the carnitine palmitoyltransferase system that is involved in initiating the transport of LCFAs into the mitochondria (Carnitine Shuttle). In order for L-carnitine to enter the mitochondrial matrix, it must first be in the form of an ester, acylcarnitine (Higdon, 2002). CPT I, the rate limiting step in fatty acid oxidation, will then enable the transfer of the long-chain fatty acids into the cytosol from coenzyme-A (CoA) to L-carnitine (Higdon, 2002). Once the carnitine is in the matrix, CPT II exchanges CoA for carnitine to produce fatty-acid CoA, which is then ready to enter fatty oxidation once more to produce energy, and the free carnitine is released back into the system to repeat the process (Carnitine shuttle).

Current studies have produced conflicting results as to whether endurance athletes need carnitine supplementation. For example, Broad and colleagues (2006) examined the dietary carnitine intake and carnitine status of endurance-trained males. Researchers recruited 14 non-vegetarian endurance-trained males (ages 18-50 years), all of which completed a seven-day weighed food record and exercise record prior to the study to determine habitual carnitine intake. Resting blood samples and urine analyses were used to determine plasma carnitine concentrations and carnitine excretion in the urine. Researchers found that there were no correlations between dietary carnitine intake and 1) plasma carnitine concentrations ($r = 0.13, p = 0.68$) 2) free carnitine concentrations ($0.36, p = 0.23$) 3) acyl carnitine concentrations ($-0.21, p = 0.50$) or 4) urinary carnitine excretion (total $-0.05, p = 0.87$; free $0.07, p = 0.81$; acyl $-0.16, p = 0.58$). The dietary records of the endurance athletes, along with the results of the study, conclude that the carnitine levels of the subjects were comparable to normal adult levels and no dietary insufficiencies were present. Furthermore, no correlation could be noted between dietary carnitine intake, dietary macro- or micronutrients, and plasma carnitine or urinary carnitine excretion, indicating the subjects were not at risk for a carnitine deficiency.

Carnitine Metabolism during Exercise

In a fed state, the liver is both an organ of glucose uptake and fat oxidation, the latter converting glucose to LCFAs, which can then be either stored in the liver or transported to other tissues (Foster, 2006). However, in a fasted state, these metabolic pathways are essentially reversed. In a fasted state, the glucose production and fatty acid synthesis in the liver cease, and fatty acid oxidation begins to take form. This mechanism is one way of providing a defense against muscle protein catabolism (Foster, 2006).

Muscle fuel selection is an important factor to consider when it comes to exercise and carnitine supplementation. Two major substrates available for energy during exercise include fat and carbohydrates. Under high-intensity, submaximal conditions ($> 70\% \text{ VO}_{2\text{max}}$), the body will utilize mainly muscle glycogen stores for fuel; however, these stores are relatively small compared to fat stores (Wall et al., 2013). Studies suggest that exercise at lower intensities (55-70% $\text{VO}_{2\text{max}}$) will enhance fat oxidation to its maximum potential in endurance-trained athletes (Achten et al., 2002). Optimizing the use of endogenous fat is an important factor to consider during endurance exercise as it may spare muscle glycogen stores (Wall et al., 2013).

One study by Hottenrott and colleagues (2012) examined the effects of high-intensity training vs. endurance training on aerobic power and body composition in 34 recreationally active men and women. Participants were randomly separated into two groups - 1) weekend group (WE), who performed two sessions of continuous endurance exercise training on the weekend, and 2) after-work-group (AW), who performed four high-intensity training sessions and an additional endurance run during the week, after work. Pre-and post-tests included heart rate monitoring, body composition (total body fat, total muscle mass, visceral fat), and a fitness assessment for cardiovascular health (VO_{peak}). For the 12-week intervention, the WE group performed 2 h 30 min of continuous endurance running, and AW performed four 30 min sessions of high intensity training and an additional 30 min endurance run. After the 12-week intervention, subjects then competed in a half marathon. Results of the study found significant decreases in both heart rate and body mass in both groups (WE and AW), however the AW group did not produce significant losses in fat free mass ($p = 0.27$) or total body mass, but there were significant decreases in total body mass, visceral fat (6.5%), and fat free mass in the WE group. Furthermore, both groups (AW and WE) significantly increased aerobic power ($p = 0.01$)

after the intervention, however, differences between groups were not observed. Overall, all subjects completed the half marathon after the 12-week intervention. This study suggests that both high-intensity and endurance exercise have the ability to improve aerobic capacity as well as body composition in recreationally trained men and women.

In addition, exercise modality plays an important role in ventilatory patterns. Generally, the metabolic demands of exercise depend highly on the mode, as well as the amount of muscle mass recruited to perform the action (Tanner, Joseph, & Stager, 2014). To date, research examining ventilatory strategies used during cycle ergometry and treadmill running are scarce. In a study by Tanner and colleagues (2014), twenty-two healthy, trained men (age 23.6 ± 5.7 years) were recruited to perform two maximal exercise tests in different modes, running and cycling. Participants were actively involved in running, cycling, or both, and were free of any cardiovascular or pulmonary disorders. On two separate testing occasions, participants reported to the lab 4 hours post-prandial, with two days in between each testing session. Each session consisted of a continuous, incremental exercise bout of running on a treadmill or cycling on an ergometer to determine maximal aerobic capacity.

Furthermore, Smith and colleagues (2008) examined the effects of glycine propionyl-L-carnitine on aerobic and anaerobic performance in 43 untrained men and women (ages 18-44). Participants performed a graded exercise test (GXT) on a motorized treadmill where expired gases were collected and VO_{2peak} and anaerobic threshold were determined. Researchers also monitored blood pressure, heart rate, respiratory-exchange ratio (RER), and perceived exertion (Borg Scale) during the GXT. Anaerobic testing consisted of participants performing a 30-s cycle sprint test on a stationary cycle ergometer attached to a computer. During testing, peak and mean power relative to lean body mass were recorded as well as fatigue rate, and total work

relative to lean body mass. All participants performed the same tests but on different days. Following the pre-intervention tests - in a randomized, double blind manner - participants were assigned to one of three conditions: Placebo (1 g cellulose), GPLC-1 (348 mg glycine + PLC at 1 g/day), or GPLC-3 (1,044 mg glycine + PLC at 3 g/day). Participants ingested three capsules two times per day for 8 weeks, then completed an identical post-intervention testing protocol. No significant differences ($P > 0.05$) were identified between or within the three groups for free, total, or acyl carnitine concentrations, or any of the performance variables. These findings suggest that GPLC supplementation has little, if any, effect on improving aerobic or anaerobic performance in untrained men and women more than aerobic exercise alone.

During physical exercise, the ratio of carnitine pools in the body is constantly shifting between various body compartments, due mainly to the esterification of the free carnitine within the muscle pool with various acyl groups (Swart, Rossouw, Loots, & Kruger, 1997). Research suggests that endurance exercise may reduce these carnitine pools in the skeletal muscle and a carnitine deficiency may ensue - carnitine supplementation attenuating this process (Swart et al., 1997). For example, Swart and colleagues (1997) examined the effect of L-carnitine supplementation on plasma carnitine levels and various performance parameters of seven male marathon athletes (age 27 ± 3 years). Each participant served as their own control, completing two progressive treadmill tests to exhaustion (0° gradient) separated by six weeks. Heart rate, oxygen consumption, and RER were measured during the test, and blood samples were drawn afterwards to determine the total carnitine (TC), free carnitine (FC), and average carnitine (AC) levels in the blood. Significant increases were found in the mean levels of TC and FC concentrations ($p < 0.05$) after L-carnitine supplementation and researchers saw a 5.68% average increase in peak treadmill running speed. Furthermore, subjects were able to maintain a higher

peak running speed, at a lower rate of O₂ consumption after the carnitine supplementation, and RER significantly decreased as well. Further research is needed but these findings support carnitine supplementation and its ability to enhance overall running performance and aerobic capacity.

Carnitine and Carbohydrate (CHO) Supplementation

The American College of Sports Medicine (ACSM) recognizes carbohydrate supplementation as an important aspect of an athlete's training and nutrition program. ACSM guidelines recommend that endurance athletes consume 2-3 grams of CHO/kg of body weight three to four hours prior to exercise, and fuel every 40-60 minutes. Furthermore, the effect of CHO on performance depends greatly on the duration and intensity of the event, as well as the training status of the athlete, the type of CHO, and the mode of exercise (Stellingwerff & Cox, 2014). There are various mechanisms that exist to explain the role of CHO and preventing fatigue, one being an oral stimulation of the central nervous system (CNS) (Stellingwerff & Cox, 2014). For example, CHO can attenuate the effects of fatigue via a direct energy contribution from CHO oxidation during longer endurance events, as muscle glycogen stores became depleted more quickly (Stellingwerff & Cox, 2014). Furthermore, research has shown that CHO supplements have provided a higher benefit to athletes compared to ingesting water alone (Jeukendrup, 2010).

Smith and colleagues (2010) examined glucose ingestion (15, 30, and 60 g/h) on endurance performance and fuel selection in 12 recreational healthy male cyclists. Peak VO₂ and onset of blood lactate accumulation (OBLA) were assessed before testing began. Subjects completed a 2-h cycling exercise at 95% OBLA followed by a simulated 20-km time trial. Subjects completed four exercise trials - ingesting 2,000 mL of one of four beverages during

each 2-h ride - each separated by at least seven days. Researchers found that compared to placebo, significant increases were seen in the time trial mean power output by 7.4%, 8.3%, and 10.7% for 15, 30, and 60 g/h, respectively.

A similar example demonstrating the positive effects of CHO on endurance performance was in another study by Smith and colleagues (2013) where the dose-response relationship of CHO and performance was observed. Fifty-one recreationally trained, healthy male cyclists or triathletes (age 28.4 ± 6.7 years) were recruited for this study. Preliminary testing was used to determine VO_{2peak} and onset of blood lactate accumulation (OBLA). Subjects performed an incremental cycling protocol consisting of a 2-h constant load ride at 95% OBLA, immediately followed by a 20-km time trial on their own bicycles stationed to a bicycle trainer. During four randomized trials, with 7 days in between each trial, subjects consumed 2,000 mL of 1 of 13 beverages (ranging from 0% - 12% CHO) and electrolytes. Beverages were consumed every 15 minutes in 250-mL portions. Results of the study were able to show that cycling at a VO_{2peak} of ~70%, along with the ingestion of a CHO beverage, significantly improved cycling performance compared to placebo during a 20-km cycling time trial. Furthermore, improvements in performance of 1.0%, 2.0%, 3.0%, 4.0%, and 4.7% were seen at 9, 19, 31, 48, and 78 g/h respectively, with 78 g/h eliciting the greatest response, as performance enhancement diminished at levels > 78 g/h. In conclusion, this study was able to demonstrate that CHO supplementation during prolonged exercise durations appear to enhance athletic performance in a curvilinear dose-response manner.

More recently, researchers have begun to look at the effects of carnitine on glucose tolerance and insulin sensitivity. Researchers believe that the mechanisms in which carnitine acts on glucose metabolism requires the action of various contributing factors - 1) enhancement of

mitochondrial oxidation of long-chain acyl-CoAs 2) modulating the activity of the pyruvate dehydrogenase complex (PDHC) and the intramitochondrial acetyl-CoA/CoA ratio and 3) altering the expression of glycolytic and gluconeogenic enzymes to name a few (Ringseis, Keller, & Eder, 2012). Current research supports the notion that an accumulation of long-chain acyl-CoAs, as well as other fatty acid metabolites, impairs insulin signaling, therefore, contributing to the increased insulin resistance in the skeletal muscle and heart (Ringseis et al., 2012).

As previously mentioned, Wall and colleagues (2011) completed a study that examined the effects of oral L-carnitine and carbohydrate ingestion on muscle carnitine concentrations and fuel metabolism during endurance exercise. Researchers were able to demonstrate that muscle carnitine concentrations could be increased (~15%) in the presence of high serum insulin levels as well as hypercarnitinaemia (550-600 μmol). On three different occasions over a 24-week period, participants underwent a 90-minute cycling protocol at various intensities (50% and 80% of $\text{VO}_{2\text{max}}$) to determine whether or not carnitine supplementation, in combination with carbohydrates, had any effect on body composition, carnitine concentrations in the muscle, or fuel metabolism. The results of the study revealed no body mass changes in the carnitine group over the 24-week period. However, when compared to the control group, plasma total carnitine (TC) concentrations increased in the carnitine group from 12 to 24 weeks, and perceived exertion was also significantly lower in the carnitine group ($p < 0.05$) compared to baseline and control ($p < 0.05$) after 24 weeks.

Short-term vs. Chronic Carnitine Supplementation

Currently, few studies can propose a standard period of time that would indicate an ideal protocol for carnitine supplementation and thus far, the daily requirement of exogenous carnitine supplementation is unknown (Broad, Bolger, & Galloway, 2006). For example, Orer and colleagues (2014) examined the effect of two different doses of acute carnitine supplementation on endurance performance in athletes. Twenty-six healthy trained males (ages 17-19 years) were recruited for the study. Using a double-blind protocol, each participant was instructed to consume either 3g (LK-3) or 4g (LK-4) of L-carnitine mixed with fruit juice 1 hour before measurements were taken. The subjects then completed a progressive treadmill test to exhaustion, where heart rate was observed during the test, and blood samples were taken afterwards to measure blood lactate concentrations. One week after the completion of the test, the athletes returned to the testing site where they underwent the same procedure, however, this time they were given placebo fluids (P-3) and (P-4). When comparing both sets of groups (LK-3 and P-3, and LK-4 and P-4) significant differences could be noted. A significant difference was found regarding heart rate and lactate (La) concentrations between the LK-4 and P-4 groups ($p \leq 0.05$) whereas heart rate and La dropped between running speeds of 8 and 16 km/h. Furthermore, significant decreases could be noted in the running speeds in both supplemented groups compared with placebos ($p \leq 0.05$).

In addition, Arazi and Mehrtash (2017) examined the effect of acute carnitine feeding on plasma glucose and lactate concentration, as well as aerobic and anaerobic capacity in elite male artistic gymnasts. Eighteen gymnasts were recruited for the study and, using a double-blind, placebo-controlled randomized protocol, subjects were divided into two groups: supplementation or placebo. Subjects were instructed to perform both an aerobic (20 m shuttle run) and anaerobic

running-based sprint (RAST) test. 90 minutes prior to the test, subjects in the supplementation group consumed 3 g of L-carnitine or placebo (maltodextrin) with 200 ml of water. Researchers collected blood samples 5 minutes before and 4 minutes after the performance trials to assess lactate and glucose. Results of this study found that, compared to the placebo group, lactate concentrations in the supplementation group were significantly lower ($p \leq 0.05$) after the aerobic (140.4 ± 15.5 mg/dl) and anaerobic (147.8 ± 12.8 mg/dl) exercise tests.

Another example of acute carnitine feeding was in a study done by Parandak and colleagues (2015) where the effects of acute carnitine ingestion on exercise-induced oxidative stress and muscle damage were examined. Participants for the study consisted of twenty-one healthy, active men. Preliminary testing included a VO_{2max} test using the Balke 15-min run test on a standardized track where participants were encouraged to give maximum effort. In a randomized, double blind, placebo-controlled manner, participants were assigned to one of two groups - carnitine (C) or placebo (P) - based on their VO_{2max} . For fourteen days, participants consumed a daily dose of either 2 capsules containing 2,000 mg L-carnitine or the placebo (2 capsules containing 2,000 mg lactose). Following the 14-day period, the same VO_{2max} test was administered to the participants under the same conditions. Results of the study revealed no changes in body composition over the two-week period, however, there were significant increases in markers of muscle damage (i.e., lactate dehydrogenase activity ($p < 0.05$), creatine kinase activity ($p > 0.05$), and total antioxidant activity ($p < 0.05$)) immediately after, as well as 2 and 24 hours post exercise in both groups. In addition, there was no significant difference between running records for the two groups (P group: 73 ± 8.2 vs C group: 71 ± 6.8 min), however, this may be due to the subjects being placed in groups possessing similar VO_{2max} values. Overall,

this study was able to demonstrate that acute L-carnitine supplementation may help to alleviate some of the side effects of short bouts of exercise, but more research is needed.

Likewise, a study by Abramowicz and Galloway (2005) compared the effects of acute vs. chronic L-carnitine L-tartrate (LCLT) supplementation on metabolic responses to steady state exercise in males and females. Twelve active, healthy subjects volunteered for the study: six males (age 25 ± 6 years) and six females (age 30 ± 5 years). Subjects were randomly divided, in a double-blind crossover design, into 3 periods: placebo (14 days of 3 g glucose/day), acute supplementation (13 days of 3 g glucose/day, plus 1 day of 3 g LCLT), or chronic supplementation (14 days of 3 g/day LCLT). Over a period of seven weeks, subjects reported to the lab on a total of six occasions where they underwent various testing. The first visit consisted of a VO_{2max} test using an incremental cycle ergometer; the second and third visits were used as familiarization trials and to determine correct workload. During the final three trials, subjects were asked to cycle for 60 minutes at 60% of their VO_{2max} . Blood samples were collected throughout the exercise protocol to determine blood lactate and plasma glycerol concentrations, and perceived exertion (6-20 Borg scale), respiratory exchange ratio (RER), and heart rate were monitored as well. The results of the study show that, over the course of the 60-minute exercise, RER decreased significantly in both genders ($p < 0.01$). In male subjects, a higher RER was observed in the chronic ingestion trial compared to placebo ($p = 0.04$), with no difference between placebo and acute trials ($p = 0.78$). On the other hand, no significant differences were seen between groups in the female population in either the acute ($p = 0.07$) or chronic ($p = 0.06$) ingestion trials. Overall, the increased RER in the male population indicated an increased reliance on CHO metabolism, with a decreased reliance on fat metabolism, throughout the 60-minute exercise protocol for both genders. The current study does not support the effect of a

single dose (2-weeks) of L-carnitine L-Tartrate, in combination with CHO ingestion, on promoting fat oxidation during exercise, however, there is an enhanced rate of CHO oxidation.

As previously mentioned, recent research has reported that chronic ingestion of L-carnitine and CHO has been shown to prolong exercise endurance as well as aid in the recovery of muscles post-exercise. For example, Wall et al. (2011) conducted a study on moderately trained male athletes (age 25.9 ± 2.1 years) to demonstrate that muscle carnitine concentrations could be increased in association with elevated serum insulin levels ($>50 \text{ mU l}^{-1}$) and hypercarnitinemia ($550\text{--}600 \mu\text{mol l}^{-1}$). The results of the study showed that muscle carnitine content was increased by 21% via dietary carnitine supplementation. Additionally, the researchers could demonstrate that carnitine had an ergogenic effect on the subjects, shown by an increased work output of 11% from basal measurements. Findings such as these reveal that carnitine feeding may have certain implications for athletic performance and suggest that muscle carnitine concentrations can be increased in individuals without deficiencies (Sahlin, 2011).

Research is currently lacking on whether carnitine supplementation can enhance fat oxidation in healthy humans; however, some researchers believe that carnitine may have additional benefits in terms of exercise recovery. To date, few studies have been able to determine a specific dose response for LC supplementation. For example, Spiering and colleagues (2007) examined the responses of criterion variables to different supplemental doses of L-carnitine L-Tartrate (LCLT). The study recruited eight healthy male weightlifters (ages 22 ± 3 years) with a year or more of squatting experience. Subjects consumed either 0 g, 1 g, or 2 g of LCLT at breakfast and lunch for 3 weeks followed by a subsequent squat exercise test. Handgrip was used to determine maximal strength of the arm musculature as well as blood draws to determine the appropriate washout period for carnitine concentrations to return to normal.

Perceived muscle soreness was also assessed at 24, 48, and 72 hours post resistance exercise (RE) test. Results showed that carnitine concentrations increased with the carnitine doses of 1 g and 2 g but a significant decrease in muscle soreness was noted in the 2 g dose compared to 1 g and the 1 g dose compared to 0 g. In conclusion, LCLT supplementation favorably affects markers of muscle damage and recovery after a bout of resistance exercise and even doses as little as 1 g per day can supply many of the same benefits associated with 2 g per day of LCLT.

L-carnitine and Fatigue

Although more research is necessary, some studies suggest that carnitine supplementation may attenuate the effects of fatigue by providing a protective effect on the blood platelets in the body that play a role in wound healing (Orer & Guzel, 2014). Carnitine is thought to play an important role in shortening the recovery process and attenuating the damage to the muscles caused during strenuous exercise (Orer & Guzel, 2014). For example, Cruciani and colleagues (2015) examined the effects of carnitine supplements on patients with HIV/AIDS in a randomized, double blind, placebo-controlled study. Thirty-five adults with advanced HIV/AIDS and moderate to severe fatigue were recruited for this study. Participants were randomly assigned to one of two groups - 3 g oral carnitine (increasing from 0.5 mg daily to 1.5 g twice daily by day 7) or placebo for 2 weeks. The primary outcomes that were measured included the physical, emotional, social and functional wellbeing of the patients, performance status, mood, serum carnitine level, serum lactate level, CD4 count, and more importantly, Brief Fatigue Inventory. Results of the study concluded that carnitine supplementation did not reduce fatigue in any of the 35 subjects. Likewise, carnitine supplementation did not have any effect on behavioral outcomes. On the other hand, plasma lactate concentrations fell in the carnitine group compared to the placebo group and plasma total and free carnitine levels increased. This study was unable to

support the use of carnitine supplementation to reduce feelings of fatigue in advanced HIV/AIDS patients with moderate to severe fatigue. Although the results of this study were inconclusive, future studies may benefit from increasing the dose/duration of the carnitine supplementation in hopes to produce significant findings.

One study done by Yu and colleagues (2011) examined the effects of a single dose of L-carnitine on the antioxidant capacity in healthy men and women (mean age 27.7 ± 4.7 years). Subjects consumed 2.0 g L-carnitine in 200 mL warm water, where venous blood samples were then taken at various time points between 0 and 24 hours after administration to determine plasma carnitine concentrations. For this study, researchers measured plasma antioxidant status in all subjects via the enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase and total antioxidative capacity (T-AOC) activity. A gradual increase in SOD concentration, as well as GSH-Px, and catalase T-AOC from 0 h to 3.5 h, whereas levels peaked at 3.5 h, and gradually returned to 0 h at the 24 h point. Overall, the increase in activity of these antioxidant enzymes suggests that a single dose of L-carnitine in healthy human subjects may be useful in treating certain cardiovascular and other chronic diseases that are associated with high levels of oxidative stress.

Female Menstrual Cycle and Performance

As females become more involved in amateur and professional sports, being familiar with how the menstruation cycle may affect performance becomes especially important. Researchers became more interested in examining the effects of the female menstrual cycle on athletic performance (Tsampoukos, Peckham, James, & Nevill, 2010) and whether or not the menstrual cycle phase could possibly hinder athletic ability. For instance, Tsampoukos and colleagues (2010) examined the effects of the menstrual cycle phase (MCP) on sprinting and the

recovery response to exercise. Fourteen highly trained female sport science students (mean age 20.1 ± 0.3 years) volunteered for the study. Subjects were eumenorrheic, were studied prior to ovulation, and refrained from taking any oral contraceptives for at least 4 months prior to the study. Two important hormones associated with the menstrual cycle – 17 B-estradiol and progesterone, were noted for this study. Once preliminary trials were done, subjects completed a performance test on three different occasions (follicular phase, just prior to ovulation, luteal phase) consisting of a 30-sec sprint with a 2-min recovery period on a non-motorized treadmill. Peak power output (PPO), mean power output (MPO), fatigue index for power (FIPO), peak speed (PS), mean speed (MS) and fatigue index for speed (FISP) were measured during the performance test and blood samples were analyzed at different time points during and after the test (rest, post-warm-up, immediately after the first sprint, immediately after the second sprint and at 5, 10, 15, 20 and 30 min during the recovery from the second sprint). Results of the trials determined that, for the 8 subjects included in the study, no significant changes in body mass could be seen ($p > 0.05$). Furthermore, no significant changes in any of the performance variables were seen, as they were unaffected by MCP ($p > 0.05$). The main findings of this study conclude that athletic performances on a 30-second sprint test were not influenced by the hormonal fluctuations of MCP or by 17 B-estradiol and progesterone, which fluctuate during the menstrual cycle. This research supports the notion that MCP likely does not affect performance in the female athlete under eumenorrheic conditions.

Another point to consider when females are readily involved in endurance training is the use of oral contraceptives. Plasma ethanol estradiol, a hormone found in many oral contraceptives, inhibits the production of the hormone 17 B-estradiol, and appears six times higher in individuals taking oral contraceptives than those that do not (Joyce, Sabapathy, Bulmer,

& Minahan, 2013). A study by Joyce and colleagues (2013) set out to determine the effect of long-term use of oral contraceptives on the endurance performance of 16 recreationally active women. The participants were separated into two groups: oral contraceptive (OC) and those who were not using oral contraceptives (CON). Once separated, baseline blood samples were taken to assess 17 B-estradiol and progesterone levels. Then, participants completed an incremental exercise test to exhaustion on an electronically braked cycle ergometer to determine peak VO_2 ($\text{VO}_{2\text{peak}}$) and anaerobic threshold (AT). The test consisted of 3 minutes of cycling at 30 W, followed by power increments at 10 W for 30 seconds until volitional exhaustion. Participants also completed four submaximal exercise tests (the first two as practice tests) that involved cycling at a cadence of 70 RPM for three work stages. During the tests, heart rate (HR), ratings of perceived exertion (RPE), pulmonary gas exchange, blood lactate concentration (La), and time to exhaustion were measured. Overall, significant differences were observed in $\text{VO}_{2\text{peak}}$ ($P = 0.03$) and VO_2 at AT ($P = 0.02$) in the CON group when compared to the OC group. However, HR, BP, and La concentrations were not significantly different between the groups. The results of the current study propose that long-term use of oral contraceptives negatively effect $\text{VO}_{2\text{peak}}$ and VO_2 at AT, but have no effect on athletic performance in recreationally trained female endurance runners.

The menstrual cycle can be divided into four phases: the menstrual phase, the follicular phase, the ovulation phase, and the luteal phase. For the most part, studies suggest that the menstrual cycle does not affect athletic performance in females; however, a study by Ubukata and Matsumura (2015) suggests that the menstrual cycle *can* be affected during these different phases. Ubukata and Matsumura (2015) suggest that injuries occur more often in females during the menstrual phase than the rest of the menstrual cycle, since estrogen and progesterone levels

are low during this time. For this study, researchers examined the relationship between the phases of the menstrual cycle and the transverse abdominis muscle (TrA). Fifteen healthy, young females (age 18.6 ± 0.9 years) with regular menstrual cycles were chosen for this study. The thickness of the TrA was measured a total of three times, using an ultrasound imaging apparatus under both resting and contractile conditions. Likewise, both the left and the right side were measured and the average thickness of the muscle on both sides used for analysis. The results of the study showed that, regardless of menstrual cycle phase, the thickness of the TrA muscle increased significantly during contraction. Overall, researchers were unable to observe any significant differences in muscle thickness of the TrA in the menstrual cycle phase; thus, muscle contraction was not affected during the fluctuation of sex hormones, indicating the TrA does not depend on the menstrual cycle.

CHAPTER 3

METHODOLOGY

Participants

Eight, healthy, recreational female endurance runners were recruited for this study. Volunteers were college students between the ages of 19 and 22 years old. The Institutional Review Board (IRB) at Georgia Southern University approved this study. The participants were informed of the possible risks involved with participating in this study and completed written informed consent. The participant's demographic information was collected, which included the number of days per week they were currently running and how long each running session typically lasted. All participants were also asked to complete a 72-hour dietary recall to assess average carbohydrate intake, as well as average carnitine intake.

Instrumentation

Participants had their weight and height assessed in the human performance lab at Georgia Southern University using a calibrated scale and stadiometer during the first week of the study. Participants also completed a 72-hour dietary recall during the first week before the first trial to assess average carbohydrate and carnitine intake. Participants were asked to maintain their current training regimen and diet throughout the duration of the study. During the first trial, participants completed a body composition assessment using the Bod Pod® and performed a timed treadmill test to exhaustion. For the BodPod body composition analysis, optimal results require participants to wear minimal clothing (spandex shorts and/or a sports bra) and a swim cap. They are then weighed on a calibrated digital scale to the nearest 20 g. Next, the participant sat in the air displacement plethysmography (ADP) chamber and be asked to breathe normally while three body measurements will be taken to assess body volume. In addition, thoracic gas

volume measurements were predicted using gender, age, and height. Measurements taken from the BodPod include: lung volume (L), body volume (L), body density (kg/L), body fat percentage, fat weight (kg), and lean weight (kg).

During the first visit, each participant was given oral and written information regarding the testing procedures. Participants performed a $\text{VO}_{2\text{peak}}$ test one week before the first trial, and were asked to complete a second $\text{VO}_{2\text{peak}}$ test one week after the final trial to verify that no changes in aerobic capacity had occurred over the course of the study. Expired gases were analyzed using the ParvoMedics TrueOne® 2400 open circuit spirometry system. This consists of a room air auto-calibration routine and a two-point gas calibration with a single gas tank (15.09% O_2 , 6.01% CO_2). The ParvoMedics TrueOne® 2400 has been shown to be an accurate and reliable device for the measurement of gas exchange variables (Crouter et al., 2006). All measurements were taken in the human performance lab at Georgia Southern University in the same session.

Procedures

Food Log Protocol: Prior to the first trial, participants were instructed to complete a 72-hour measured dietary recall (one weekend day and two week days). Participants downloaded the MyFitness Pal app that is available on both iPhone and Android devices. The dietary recall was analyzed for an estimate of baseline carbohydrate intake, as well as total daily macronutrient intake. An estimate of carnitine consumption was also determined using a calculation used in previous research (Rebouche, 1999).

Exercise Protocol: One week before the trial, each participant's maximal oxygen uptake ($\text{VO}_{2\text{peak}}$) was measured while participants ran on a treadmill to determine baseline measurements. Upon arrival, height (cm) - measured using a stadiometer, weight (kg) - measured

using a calibrated scale, and body mass index (kg/m^2) was determined. Participants were instructed to 1) wear comfortable, loose-fitting clothing, 2) refrain from consuming any alcohol, tobacco, or caffeine for at least 12 hours prior to taking the test, 3) refrain from participating in any strenuous physical activity the day before, as well as the day of the test, 4) get an adequate amount of sleep the night before the test (6-8 hours).

Prior to testing, participants were fitted with headgear, a mouthpiece and a nose clip. Heart rate was monitored continuously throughout the protocol by a Polar® Heart Rate monitor with a sensor located on the chest. Participants completed a 3-5 minute warm-up at a self-selected speed at a 0° grade. The participant was informed that this self-selected speed should be similar to a light jog, nothing too taxing on the runner. The speed the participant chose to run at determined the starting point for their exercise protocol. After the initial warm-up, the test began and was comprised of 2-minute stages. In each stage, speed was raised by 1.0 mph and after the third stage, grade increased by 2% per stage. Through the duration of the test, various criteria was monitored including: VO_2 , heart rate (HR), respiratory exchange ratio (RER), and rate of perceived exertion (RPE). RPE (Borg Scale 6-20) was taken at the end of each stage. Criteria for ending the test included: (1) a plateau in oxygen uptake (2) maximal heart rate was reached (3) $\text{RER} > 1.15$ (4) volitional exhaustion (the point at which the participant voluntarily stopped the exercise). The test was discontinued if all test criteria were achieved or when the participant chose to stop.

One week after the pre-testing, subjects returned for the first trial, performing a progressive treadmill test (0° gradient) to exhaustion. Participants began by running at 8 km/h and the running speed increased every three minutes by 2 km/h until 16 km/h was reached or exhaustion was achieved. Thereafter, the speed increased every two minutes by 1 km/h until

exhaustion, at which point the test was then terminated. Peak running speed was measured at the highest running speed (km/h) the athlete could maintain for 60 seconds during the exercise test. During the duration of the test, heart rate and perceived exertion were monitored and time to fatigue was also noted.

Supplementation Protocol: Participants were divided into two groups based on similar VO_{2peak} values and then randomly assigned to one of two treatment groups: LC or LC+CHO. Subjects were then instructed to consume their treatment two times per day for 42 days - the first supplement 30 minutes before breakfast, and the second supplement 4 hours later or 20-30 minutes before exercise, per the manufacturer's directions on the bottle. Volunteers were requested to record any side effects associated with supplementation over the 8-week protocol. The composition of the supplements is outlined below.

L-Carnitine (LC) Group: 1 scoop (1.30 g) carnitine powder (Gaspari Nutrition ® Carnitine Tartrate [as Carnipure™] in the flavor Pineapple) mixed with 200 mL of water containing 0 g CHO, 0 g protein, 0 g fat; **Zero Calories per serving.**

L-Carnitine + Carbohydrate (LC + CHO) Group: 1 scoop (1.30g) carnitine powder (Gaspari Nutrition ® Carnitine Tartrate [as Carnipure™] in the flavor Pineapple) and 10 g NOW! Dextrose Powder mixed with 200 mL water. The supplement will contain 9 g CHO, 0 g protein, 0 g fat; **35 calories per serving.**

Supplements were assembled by the researcher in advance and given to participants on the day of the first exercise session, and again when they returned for the second exercise session. Directions for supplement use were given verbally and were also written on the container that held the supplements.

Study Time Line

Week 1: An informational meeting was held with the participants, during which, the experimental procedures of the three sessions were reviewed. The timeline for the experiment was explained to the participants during this time. Each participant read and sign the informed consent. The researcher clearly explained to the participants that study participation was voluntary. During week 1, the researcher took anthropometric (height and weight) measurements of the participants. The participants were also provided with instructions on how to record the measured food intake for the dietary recall. Also during week one, participants completed a VO_{2peak} test to assess maximal oxygen uptake. Subjects were then divided into two groups based on VO_{2peak} values, and then randomly divided into one of the two treatment groups.

Group 1: L-carnitine

Group 2: L-carnitine + carbohydrate

Week 2-4: Measured food logs were shared with the researcher on the first day of testing to assess total carbohydrate and carnitine intake. Participants completed a progressive treadmill test to exhaustion as well as a body composition assessment (Bod Pod). Participants began the supplementation regimen the day after the first exercise session.

Week 5-6: Participants completed the second session of treadmill testing and body composition assessments and continue their daily supplementation regimen.

Week 7: Subjects completed the third, and final, session of treadmill testing and body composition assessments and supplementation ended.

Week 8: Subjects returned for final VO_{2peak} testing.

Statistical Analysis

Three one-way ANOVAs with repeated measures were conducted to compare the effect of the treatment (LC or LC + CHO) on the participants' average time to fatigue, average body composition, and average RPE. It was assumed that participants would return for all required exercise protocols; would take their required dosages of the supplement given; would take their required supplements at the correct time and frequency throughout the day; participants would not be taking any performance enhancing drugs at the time of the study; would continue with their current diet and training regimen throughout the duration of the study; the Bod Pod® is a reliable and valid measure of body composition; and the ParvoMedics TrueOne® 2400 proves to be an accurate and reliable device for the measurement of gas exchange variables

CHAPTER 4 RESULTS

For average time to fatigue, the analysis revealed no significant interactions or main effects for time and treatment, Wilks' Lambda = .33, $F(2,4) = 4.02$, $p = .11$, $\eta^2_{\text{partial}} = .668$. In addition, no significant differences were seen between or within subjects for body composition (%FFM, Wilks' Lambda = .74, $F(2,4) = .72$, $p = .54$, $\eta^2_{\text{partial}} = .26$; FFM, Wilks' Lambda = .41, $F(2,4) = 2.89$, $p = .17$, $\eta^2_{\text{partial}} = .59$; FM, Wilks' Lambda = .87, $F(2,4) = .31$, $p = .75$, $\eta^2_{\text{partial}} = .13$ or %FM, Wilks' Lambda = .76, $F(2,4) = .64$, $p = .57$, $\eta^2_{\text{partial}} = .24$) or RPE, Wilks' Lambda = .97, $F(2,4) = .06$, $p = .95$, $\eta^2_{\text{partial}} = .03$.

Participant Characteristics

Seven female participants completed the study. One subject was removed from the study after failure to adhere to required criteria for the study. All other participants reported to the study for all testing sessions. Descriptive statistics are summarized in Table 1.

Participants performed a $VO_{2\text{peak}}$ test before experimental testing began. Participants were then divided into groups based on similar $VO_{2\text{peak}}$ values, as this indicates similar levels of fitness. Participants in both groups were tested before and after the 8-week supplementation and exercise protocol to assess pre- and post-test values. Table 2 shows the results of the pre- and post- $VO_{2\text{peak}}$ test for both supplement groups.

Participants' time to fatigue was recorded after each progressive treadmill test. An ANOVA with repeated measures was used to compare time to fatigue between and within both supplement groups before and after the 8-week supplementation period. Results of the analysis revealed no significant interactions or main effects for time and treatment ($p = .11$), however, analysis revealed a large effect size ($\eta^2_{\text{partial}} = .668$).

In addition to the progressive treadmill test, participants were required to have body composition measurements performed using the BodPod body analyzer. Body composition measurements were performed before each progressive treadmill test and included percent fat free mass (%FFM), fat free mass (FFM), fat mass (FM), and percent fat mass (%FM). An ANOVA with repeated measures was used to compare body composition between and within both supplement groups before and after the 8-week supplementation period. The ANOVA revealed no significant differences between subjects for body composition (%FFM $p = .54$; FFM $p = .17$; FM $p = .75$; or %FM $p = .57$) but did show a large effect size for all components of the body composition assessment ($\eta^2_{\text{partial}} = .26$, $\eta^2_{\text{partial}} = .59$, $\eta^2_{\text{partial}} = .13$, $\eta^2_{\text{partial}} = .24$, respectively). Tables 4, 5, 6, and 7 display the data collected from each assessment.

Furthermore, rating of perceived exertion was recorded during each progressive treadmill test. An ANOVA with repeated measures was used to compare ratings of perceived exertion between and within both supplement groups before and after the 8-week supplementation protocol. Table 8 shows the results of the rating of perceived exertion for both supplement groups for each exercise protocol. Results of the analysis revealed no significant differences between or within subjects for both groups ($p = .95$).

In order to get an idea of the average number of carbohydrates and carnitine consumed by each participant, average carbohydrate and carnitine intake were assessed using a 72-hour dietary recall. Details of the recall are outlined in Table 9 and 10.

Table 1*Summary of information of subject age, weight, and other contributing factors (n = 7)*

Variable	Mean \pm SD
Age (years)	20.28 \pm 1.25
Weight (kg)	140.85 \pm 19.33
Height (cm)	171.42 \pm 7.63
Days run/week	4.78 \pm 0.80
Time run/day (min)	37.92 \pm 13.72
Average calories/day (kcal)	1675.85 \pm 474.61
Average carnitine intake/day (mg)	13.42 \pm 10.69
Average carbohydrate intake/day (g)	181.44 \pm 56.94

Table 2*Average VO_{2max} values for both treatment groups before and after supplementation(n=7)*

Variable	Mean \pm SD
LC (Pre)	38.26 \pm 1.05
LC+CHO (Pre)	45.83 \pm 2.20
LC (Post)	41.80 \pm 1.76
LC+CHO (Post)	45.50 \pm 0.85

Table 3*Average Time to Fatigue for Trial 1, 2, and 3 for both supplement groups(n=7)*

	Treatment	Mean	Std. Deviation	N
Time to Fatigue Session 1 (min)	LC	7:49	2:02	4
	LC+CHO	11:31	0:51	3
	Total	9:24	2:29	7
Time to Fatigue Session 2 (min)	LC	7:54	1:18	4
	LC+CHO	10:42	0:48	3
	Total	9:06	1:48	7
Time to Fatigue Session 3 (min)	LC	8:19	1:20	4
	LC+CHO	11:31	0:45	3
	Total	9:27	1:55	7

Table 4*Average Percent Fat Free Mass for Trial 1, 2, and 3 for both supplement groups (n=7)*

	Treatment	Mean	Std. Deviation	N
Percent Fat Free Mass Session 1	LC	74.83	5.95	4
	LC+CHO	80.40	6.19	3
	Total	77.21	6.27	7
Percent Fat Free Mass Session 2	LC	75.38	6.78	4
	LC+CHO	81.47	6.55	3
	Total	77.99	6.92	7
Percent Fat Free Mass Session 3	LC	75.38	5.26	4
	LC+CHO	81.17	7.26	3
	Total	77.43	6.16	7

Table 5*Average Fat Free Mass for Trial 1, 2, and 3 for both supplement group (n=7)*

	Treatment	Mean	Std. Deviation	N
Fat Free Mass Session 1 (kg)	LC	55.90	26.06	4
	LC+CHO	54.90	4.65	3
	Total	55.47	18.63	7
Fat Free Mass Session 2 (kg)	LC	57.33	23.60	4
	LC+CHO	55.57	3.57	3
	Total	56.57	16.84	7
Fat Free Mass Session 3 (kg)	LC	44.98	4.12	4
	LC+CHO	54.83	3.65	3
	Total	49.20	6.38	7

Table 6*Average Fat Mass for Trial 1, 2, and 3 for both supplement groups (n=7)*

	Treatment	Mean	Std. Deviation	N
Fat Mass Session 1 (kg)	LC	14.98	4.75	4
	LC+CHO	13.83	6.48	3
	Total	14.49	5.07	7
Fat Mass Session 2 (kg)	LC	18.40	7.12	4
	LC+CHO	13.13	6.81	3
	Total	16.14	6.98	7
Fat Mass Session 3 (kg)	LC	14.80	4.27	4
	LC+CHO	14.10	7.41	3
	Total	14.50	5.25	7

Table 7*Average Percent Fat Mass for Trial 1, 2, and 3 for both supplement groups (n=7)*

	Treatment	Mean	Std. Deviation	N
Percent Fat Mass Session 1 (%)	LC	25.18	5.95	4
	LC+CHO	19.60	6.19	3
	Total	22.79	6.27	7
Percent Fat Mass Session 2 (%)	LC	24.63	6.78	4
	LC+CHO	18.60	6.67	3
	Total	22.04	6.94	7
Percent Fat Mass Session 3 (%)	LC	24.63	5.26	4
	LC+CHO	19.83	7.26	3
	Total	22.57	6.16	7

Table 8*Average Rating of Perceived Exertion for Trial 1, 2, and 3 for both supplement groups (n=7)*

	Treatment	Mean	Std. Deviation	N
Rating of Perceived Exertion Session 1	LC	15.00	1.63	4
	LC+CHO	18.00	1.73	3
	Total	16.29	2.22	7
Rating of Perceived Exertion Session 2	LC	16.25	1.50	4
	LC+CHO	17.00	.00	3
	Total	16.57	1.13	7
Rating of Perceived Exertion Session 3	LC	16.50	1.00	4
	LC+CHO	17.00	.00	3
	Total	16.71	0.76	7

Table 9*Average carbohydrate intake (g) prior to the start of the study (n=7)*

Group	Mean ± SD
LC	163.72 ± 72.37
LC+CHO	204.86 ± 20.77

Table 10.*Average carnitine intake (mg) prior to the start of the study (n=7)*

Group	Mean ± SD
LC	16.50 ± 14.01
LC+CHO	9.30 ± 2.08

CHAPTER 5

DISCUSSION

One of the many functions of carnitine is its role in the translocation of long-chain fatty acids into the mitochondrial matrix for subsequent β -oxidation (Stephens, Constantin-Teodosiu, & Greenhaff, 2007). Over the last decade, researchers have been investigating the effect of carnitine supplementation as an ergogenic aid, with the idea that increasing carnitine availability in the muscle would increase fat oxidation and spare muscle glycogen stores, thus delaying the onset of fatigue. However, limited research has been able to support the use of carnitine supplementation to enhance athletic performance, and previous research has shown that carnitine feeding has no effect on muscle carnitine stores in the body. As previously mentioned, there is limited research on carnitine supplementation, particularly with carnitine in combination with carbohydrates. The purpose of this study was to examine the effect of L-carnitine, in combination with carbohydrates, on body composition and overall athletic performance in recreational female endurance athletes. The results of this study revealed no significant interactions or main effects for time and treatment for time to fatigue. In addition, no significant differences were seen between or within subjects for body composition.

Previous research has shown that L-carnitine supplementation can affect performance and fatigue in endurance athletes (Karahana et al., 2010; Stephens et al., 2013; Arazi & Mehrtash, 2017). To date, studies investigating the effect of LC supplementation on exercise performance have used gas-analysis techniques as well as cycle ergometry to evaluate performance variables, as exercise modality plays a role in ventilatory patterns. A study by Tanner, Duke, and Stager (2014) examined the effect of different modes of exercise on ventilatory patterns. Data collection showed that, although no observed differences were seen in ventilation (V_E) between running

and cycling during the maximal exercise test, different breathing patterns were observed. This difference in breathing pattern is important to the current study to distinguish the physiological differences between treadmill running versus biking, as the current study utilized the treadmill in assessing exercise performance.

Some studies have suggested that athletes are able to withstand higher workloads at lower levels of oxygen consumption during exercise after a single dose of L-carnitine (Vecchiet, Di Lisa, & Pieralisi, 1990). The function of carnitine during mitochondrial metabolism may be a contributing factor to the overall increase in effectiveness of oxygen utilization, as other studies have seen decreases in respiratory exchange ratio (RER) values during VO_{2max} testing during L-carnitine supplementation (Wyas, Ganzit, & Reizi, A, 1990). For example, Swart and colleagues (1997) examined the effect of L-carnitine supplementation on plasma carnitine levels and various performance parameters of male marathon athletes. After six weeks of L-carnitine supplementation, the results of the study revealed a 5.68% average increase in treadmill running speed compared to baseline values, and a decrease in average oxygen consumption and heart rate. In addition, a decrease in RER values were observed when compared to baseline values, which indicates an increased reliance on lipid oxidation as a fuel source during aerobic exercise.

One of the aims of the current study was to assess average time to fatigue for each timed exercise trial and, unlike Swart and colleagues (1997), the current study did not find any significant differences in those measurements between or within participants. These results could be due to participants becoming accustomed to performing the exercise protocol or participants possessing similar levels of fitness in each group. Furthermore, the participants used in the study by Swart and colleagues (1997) were highly trained, triathlon athletes, whereas the participants used in the current study were recreational, college-aged endurance runners.

In addition, Orer and Guzel (2014) examined the effects of two different doses of L-carnitine on the endurance performance of male soccer players, aged 17-19 years. Based on blood samples obtained from participants, the researchers found differences in running speeds between groups at different lactate concentrations (2, 2.5, 3, 3.5, and 4 mmol/L⁻¹), whereas fatigue occurred at a slower rate in both groups after carnitine supplementation compared with the placebo trial. The current study was unable to collect blood samples for analysis, thus preventing the examination of lactate concentrations in the blood. The findings from the study by Orer and Guzel (2014) showed that plasma lactate concentrations decreased after L-carnitine feeding, suggesting that carnitine may have the potential to help prevent muscle damage caused by exercise. Similarly, Arazi and Mehrtash (2017) also noticed significantly lower lactate levels in their supplementation group compared with placebo when examining acute L-carnitine supplementation on blood lactate, glucose, and anaerobic and aerobic performance in elite male artistic gymnasts. Workload is closely related to individual differences seen in lactate concentrations during exercise performance, therefore, future studies should determine which running speed at which heart rate an athlete reaches their anaerobic threshold.

As mentioned previously, some studies have evaluated rating of perceived exertion (RPE) to gauge perceived effort of participants (Orer & Guzel, 2014; Swart et al., 1997; Wall et al., 2011) but have found conflicting results (Spiering et al., 2007; Wachter et al., 2002) when using this as a method of perceived work output. For example, Abramowicz & Galloway (2005) examined the effect of acute versus chronic L-carnitine supplementation on twelve moderately trained athletes. Results of the study found no significant differences between trials in rating of perceived exertion in either males or females. In contrast, Wall et al., 2011 found that after 24 weeks, perceived exertion was lower in the supplement group compared to the control group.

Similar to the study by Abramowicz & Galloway (2005), the current study protocol utilized RPE to assess how hard participants believed they were working and participants in the current study were adapted to endurance-type training, which may have affected how hard they perceived they were working. This could potentially be the reason as to why the current study revealed no significant differences in rating of perceived exertion between or within groups for RPE.

Overall, observing lactate concentrations would have been an ideal way to measure how much participants adapted to the work they were performing. Experienced runners will exhibit lower blood lactate levels when compared with untrained runners, making it a good indicator of training intensity because it does not require the use of heart rate or expired gases (Goodwin, Harris, Hernandez & Gladden, 2007). Future studies would benefit from collecting blood samples to better assess lactate concentrations, and ultimately, levels of fatigue.

In addition to reducing levels of and time to fatigue, a popular claim associated with carnitine is that it aids in fat loss and helps prevent weight gain. Similar to the current study, previous studies involving L-carnitine supplementation have concluded that L-carnitine has little effect on body composition (Wutzke & Lorenz (2004); Broad, Maughan, & Galloway (2011)). For instance, Kruszewski (2011) examined the effect of L-carnitine supplementation, in combination with body building training, on body composition in beginner body builders using bioelectrical impedance analysis (BIA). Results of the study concluded that no significant changes were seen in the fat and water content of the participants after five weeks of L-carnitine supplementation. Like their study, the current study found no significant differences in body composition between or within subjects in both groups after eight weeks of L-carnitine supplementation. Furthermore, unlike the study by Kruszewski (2011), the current study used

recreationally-trained female endurance runners who met the ACSM guidelines of participating in at least 150 minutes of physical activity three to five times per week. They were also accustomed to performing endurance exercise, and therefore their fitness levels were most likely higher than the participants used in the study by Kruszewski (2011).

The current study used the Bod Pod body analyzer to assess body composition, whereas previous studies typically utilize BIA or skinfolds for body composition measurements (Broad, Maughan, & Galloway, 2008). This difference in instrumentation may explain the lack of significant changes seen in body composition in the current study. In addition, participants may have violated some of the assumptions of the study by not fasting for a minimum of two hours before their body composition measurement or performing strenuous exercise prior to testing, which may have affected the results. Likewise, some participants had to be tested in the Bod Pod multiple times to get an accurate reading, as the data was not always consistent. Both the current study and the study by Kruszewski (2011) demonstrated that the claims made by manufacturers about L-carnitine may not always be true, however, L-carnitine dosing is very individualized and may be dependent upon the fitness and experience level of the individual (Kruszewski, 2011) to elicit any effect.

Although previous studies have not supported the use of carnitine as a fat loss supplement, some more recent studies have shown the potential of L-carnitine supplementation to prevent increases in fat accumulation (Stephens et al., 2013); Wall et al., 2011). For example, Stephens and colleagues (2013) examined the effect of twelve weeks of L-carnitine feeding on energy expenditure and body fat accumulation in recreationally active males. Unlike the current study, results of their study showed no change in body composition in the LC group before or after supplementation, whereas the body composition of the control group increased in every

participant after twelve weeks. In addition, when examining the effects of L-carnitine supplementation in combination with carbohydrates on muscle carnitine concentrations and fuel metabolism during endurance exercise, Wall and colleagues (2011) found no changes in body mass in the carnitine group over a period of 24 weeks. They also noticed a 2.4 kg increase in body mass after the 12-week period in the control group when compared to baseline. Compared to the current study, the Wall (2011) study protocol extended a period of 24 weeks, whereas the current study supplementation period was only 8 weeks. In addition, participants in their study were male athletes training at least 3-5 times per week, performing triathlon-type exercise (biking, running, swimming). Previous research with carnitine supplementation suggests that longer supplementation periods have shown to be more successful in finding significant changes when examining body composition (Wall and colleagues, 2011; Stephens and colleagues, 2013).

The study by Wall and colleagues (2011) also incorporated roughly 600 additional calories (80 g CHO) into the diets of their participants, while the current study protocol only included an additional 70 calories (22 g of carbohydrates). Participants in their study were informed of the caloric intake and told to account for it in the diet, but the low number of calories coming from carbohydrates in the current study protocol was most likely too small to elicit any type of physiological response such as weight gain. Unlike the studies by Wall (2011) and Stephens (2013), the current study reported overall caloric intake using a 72-hour dietary recall which isn't always accurate.

Swart and colleagues (1997) accounted for overall caloric consumption (energy from calories, carbohydrates, lipids, and protein) throughout the entire duration of their study, whereas the current study only had participants report average carbohydrate and carnitine intake over a 72-hour period. Determining percentage of calories coming from lipid sources would be an

important factor to consider in future studies since lipids must be available for carnitine supplementation to be of any value during endurance exercise. Furthermore, it must be possible for those lipids to be utilized, which is also achieved through lower-intensity exercise. As mentioned previously, dietary carnitine is predominately obtained through animal protein sources and the participant's diets in the current study, as well as in the study by Swart (1997), were more carbohydrate heavy, possibly resulting in a lower level of free carnitine in the body and thus, a slight deficiency.

Future studies may benefit from having participants record daily food logs throughout the duration of the study to keep track of energy consumed from macronutrients, as this is an important factor of overall lipid utilization and would be a more accurate way of determining caloric intake. In addition to reporting overall caloric intake, the current study protocol instructed participants to maintain their current diet and exercise regimen throughout the study protocol but societal and psychological factors may have prevented participants from doing so. For instance, Spring Break fell in the middle of the study protocol and many participants left for vacation during that time. This made it difficult for the researcher in the current study to make sure participants were still maintaining their normal routine and taking the supplements accordingly.

In conclusion, this study found that eight weeks of carnitine supplementation, in combination with carbohydrates, did not increase work output or decrease feelings of fatigue in female recreational endurance runners. In addition, eight weeks of carnitine and carbohydrate supplementation did not have a significant effect on body composition or rating of perceived exertion. This study attempted to simulate routine diet and exercise conditions for this specific population (college-aged recreational runners) during the supplementation protocol. Participants

in this study were college-aged recreational runners, therefore psychological and societal factors enhanced the possibility of diverging from normal diet and exercise routine, limiting their intake of the supplement and unknowingly altering body composition.

Furthermore, the current study had a small sample size ($n=7$), which could have been attributed to the limited inclusion criteria initially set forth by the researcher. The small sample size prevented the use of a control group in addition to the two supplement groups. Having a control group and an experimental group allows the researcher to manipulate one aspect of the study and allows the researcher to see if the group getting the treatment behaves any differently from the group not receiving the treatment (Tetyana, 2012). Having a control group is important if the researcher wants to assess the impact of a certain treatment, thus future studies may benefit from having this group. Like mentioned previously, future studies would also benefit from obtaining blood samples from participants to analyze carnitine concentrations in the muscle as well as blood lactate concentrations after ingestion of the L-carnitine and carbohydrate supplement and exercise performance.

Currently, many studies have been unable to support the use of carnitine supplementation to enhance athletic performance; however, these conclusions have been drawn based on small sample sizes, short duration of supplementation, type of supplementation, and lack of control of diet before and after supplementation (Abramowicz & Galloway, 2005). Despite the limitations in this study, the results suggest that consumers should be wary of the claims made by supplement companies that state L-carnitine helps prevent weight gain and promotes fat loss, as the data in this study suggest otherwise.

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