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Effect of Carbohydrate Supplement on Cardiovascular and Metabolic Responses to Dual Concurrent Stressors

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Effect of carbohydrate supplement on cardiovascular and metabolic responses to dual concurrent stressors

By

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Chronic psychological and physiological stress is linked to a high prevalence of cardiovascular disease (CVD). Potentially dangerous cardiovascular responses (e.g., exacerbated cortisol production) can occur with high levels of stress and chronic hypercortisolemia is associated with CVD. The ingestion of carbohydrate (CHO) prior to physical stress may attenuate cortisol responses to stress. The purpose of this project was to investigate the potential effect of CHO ingestion on cortisol production and responses to concurrent stress challenges. Sixteen apparently healthy non-smoking men 21-30 years old participated in a randomized, cross-over, double blind, placebo controlled trial. Participants were tested on four separate sessions. In session 1, general procedures were explained, and participants provided written informed consent as well as a health history questionnaire. Anthropometric data were obtained and participants performed a VO_2 peak test during session 2, as well as a 90 sec familiarization session with the mental stress challenges. During the third and fourth sessions, either a 6.6% CHO solution or non-CHO control beverage (water containing non-caloric ingredients tasting like the CHO beverage) was randomly assigned and orally ingested at 0.6g/kg body weight 15 min

prior to performing a dual-concurrent-stress (DCS) challenge. The DCS procedure consisted of physical stress (i.e., exercise) combined with computerized mental stress tests of color word associations and arithmetic. Ten mL of blood were obtained at each blood draw: 70, 40, and 15 min prior to the start of exercise, immediately at onset of exercise, 10, 20, 30, and 35 min during exercise, and 15, 30, 45, and 60 min during a post exercise recovery period. There was a significant main effect for treatment regarding mean cortisol production, and the DCS challenge was effective at increasing anxiety and acting as an effective stressor. Mean cortisol production was consistently lower during and after DSC. This is a potential beneficial implication for individuals that work in high-stress conditions. These findings support a prevention based approach to address the high prevalence of CVD among individuals and others working in high-stress occupations such as firefighters.

DEDICATION

This dissertation is dedicated to my family for providing continual support and motivation in various ways. My parents Neil and Debi have supported me through my entire life. A journey of one thousand miles begins with a single step and my parents were the ones that pushed me to take the initial step, as they were the reasons I initially transitioned into college upon high school graduation. I would have never made it this far without their persistent financial and emotional support. During the later years of graduate school, the birth of my two children Jase and Nyla has been the sole source of motivation and inspiration. I am also very lucky to have continual support and motivation from my best friend and life partner, Brooke. I love you all and would like to thank you for supporting me, as this would not have been possible without all of you. I am very thankful to have such a well-rounded, supportive and loving family.

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CHAPTER I

INTRODUCTION

The leading cause of death among firefighters is cardiovascular disease (CVD) (Kales, Soteriades, Christoudias, & Christiani, 2003; Soteriades, Smith, Tsismenakis, Baur, & Kales, 2011). Ninety percent of all deaths from CVD are attributed to coronary heart disease (CHD) (Kales, Soteriades, Christophi, & Christiani, 2007). Chronic stress may contribute significantly to atherosclerosis and the high CHD prevalence (Kumari et al., 2003). Several studies have attributed *stress* to elevated CVD risk (Carey, Al-Zaiti, Dean, Sessanna, & Finnell, 2011; Lusa, Häkkänen, Luukkonen, & Viikari-Juntura, 2002; Soteriades et al., 2011). While several reports suggest that exercise interventions may serve a cardioprotective effect (Awobajo, Olawale, & Bassey, 2013; Kozey Keadle et al., 2014; Riedel et al., 2014), a solution that is less demanding in terms of time and physical exertion is more practical. Research has linked chronic stress of both psychological and physiological origins to a high prevalence of CVD and when these conditions are duplicated in a controlled environment, potentially dangerous cardiovascular responses (e.g., exacerbated cortisol production) have been reported (Webb et al., 2008; Webb et al., 2011; Webb, Garten et al., 2011). Chronic hypercortisolemia is strongly associated with CVD (Huang, Webb, Zourdos, & Acevedo, 2013) as well as oxidative stress and hyperlipidemia (Devaki, Nirupama, & Yajurvedi, 2013).

Several investigations have demonstrated that the ingestion of carbohydrates (CHO) prior to the physical stress can reduce cortisol responses to stress (Anderson, Bryden, Polansky, & Thorp, 1991; Gomes, Moreira, Coutts, Capitani, & Aoki, 2014; de Oliveira Quirino, Gonçalves, de Oliveira, dos Santos, & Silva, 2012; McAnulty et al., 2003; Nehlsen-Cannarella et al., 1997; Nieman, 1998; Nieman et al., 1998). However, to our knowledge this treatment has not been tested under concurrent stress conditions. Alleviating chronic hypercortisolemia can potentially have tremendous benefits for firefighters in terms of improving cardiovascular health.

The effects of CHO intake on exercise performance has been extensively evaluated and supported as ergogenic (Smith et al., 2010; Sugiura & Kobayashi, 1998). Of greater relevance to this project is the potential ability of CHO ingestion to have an effect on the production of metabolic and cardiovascular responses to concurrent mental and physical stressors. The combination of these stressors results in exacerbated cortisol production (Webb et al., 2008; Webb, Fabianke-Kadue et al., 2011; Webb, Garten et al., 2011) and may impair vascular functioning as well as increase platelet aggregation (Webb, Garten et al., 2011). These mechanisms may contribute to increased prevalence of CVD among active duty firefighters (Kales et al., 2003; Kales et al., 2007; Melius, 2001; Smith et al., 2012). A higher level of cardiovascular fitness may serve to better prepare an individual for dual stress challenges (Acevedo et al., 2006; Webb et al., 2013). However, several investigations have reported that CHO intake can improve cardiovascular responses to both psychological (Sihvola et al., 2013) and physical stress (Anderson, Hoffman, Balon, Sinkey, & Mark, 1991; McAnulty et al., 2003; Nehlsen-Cannarella et al., 1997; Nieman, 1998). It has also been reported that high CHO diets

may allow for some level of protection against oxidative stress (Moller, Wallin, & Knudsen, 1996) which has been shown to result from concurrent stress challenges (Webb, Garten et al., 2011). The purpose of this project was to investigate the potential effect of exogenous CHO ingestion on cortisol production and cardiovascular responses to concurent stress challenges. These findings can serve as a prevention based approach to address the high prevalence of CVD among firefighters in the U.S.

CHAPTER II

LITERATURE REVIEW

Introduction to Stress

Organisms are constantly challenged to maintain equilibrium or homeostasis, as threatened by environmental factors (Stratakis & Chrousos, 1995). An inability for an organism to maintain homeostasis could be threatening to genetic functioning (Dobson & Smith, 2000) as well as the ability to sustain life (Selye, 1950). The concept of *stress* was originally coined by Hans Selye, and defined as a "disease of adaptation" (Selye, 1950). The initial defense against such disruptions is reflected by an organism's non-specific attempt to maintain a steady state (Stefano, Fricchione, & Slingsby, 2001). A disturbance to homeostasis in humans can be presented from a series of biochemical, physiological, and behavioral changes that may be affected by responses from neurotransmitters, peptides, or steroid hormones. However, the term "stress" or "strain" can also be discussed in terms of tension or pressure as induced by external forces which may result in deformation of an object (Le Fevre, Matheny, & Kolt, 2003; Selye, 1950). For the purpose of consistency and facilitating the scope of this review, the term "stress" will be operantly defined as any psychological or physiological stressor acting on an individual that induces a possible acute or chronic disruption of allostatic homeostasis (Juster, McEwen, & Lupien, 2010; McEwen, 2005) as maintained by the hypothalamic-pituitary-

adrenal (HPA) and sympathoadrenal (SA) axes (Le Fevre et al., 2003; Stratakis & Chrousos, 1995).

The purpose of the stress response (i.e., physiological response to stress) is to serve a functional fight or flight mechanism (Stratakis & Chrousos, 1995) that will later be described in further detail. This review will initially address two separate categories of stressors: psychological and physiological. However, several investigations have shown that a combination of these types of stressors can result in potentially dangerous cardiovascular events including exacerbated activation of the SA (Acevedo et al., 2006; Huang, Webb, Evans et al., 2010; Huang, Webb, Garten et al., 2010; Roth, Bachtler, & Fillingim, 1990; Rousselle, Blascovich, & Kelsey, 1995; Szabo, Peronnet, Gauvin, & Furedy, 1994; Webb et al., 2008; Webb et al., 2010) and HPA (Webb et al., 2008; Webb, Fabianke-Kadue et al., 2011; Webb, Garten et al., 2011; Webb et al., 2013) axes compared to that seen from a single stressor.

Typical physiological and psychological responses to stress include increased heart rate, respiration and perspiration rate, dry lips, queasy stomach, feeling anxious or tense, or the desire to leave the stress situation (Soewondo, 1996). Psychological stressors can originate from a variety of sources such as work or academic demands (Valdez, Chavez, & Woulfe, 2013), busy life or work schedule, social or economic stressors such as unemployment or cost of living, uncertainty, or changes in life (Soewondo, 1996). Stress can result in either a positive or negative outcome and as such, the terms eustress and/or distress are respectively applied (Le Fevre et al., 2003). The concept of eustress is usually associated with situations where stress is acute and transient, rather than chronic and excessive. The relationship between stress and physical and/or mental performance

represents an inverted "U," such that a moderate level of stress is associated with optimal performance (Benson & Allen, 1980). Beyond the optimal level of physiological arousal, performance tends to decrease (Le Fevre et al., 2003); hence, the relationship between *moderate* stress and eustress; and/or *chronic* stress and distress.

Eustress is associated with an initial perception of fear or anxiety and may potentially lead to a beneficial outcome such as improved academic/work performance (Le Fevre et al., 2003), or some type of pleasurable or positive perception of the outcome (Edwards & Cooper, 1988; Harris, 1970). Distress is associated with chronic stress and increased prevalence of health risks or decreased performance (Chandola, Brunner, & Marmot, 2006; Juster et al., 2010). It should be noted that the distinction between eustress or distress can potentially be made by the individual's *perception* of the demands of the particular stressor (Le Fevre et al., 2003). Merely the perception to stressors can tremendously influence an individual's risk for developing stress related diseases (Lupien, McEwen, Gunnar, & Heim, 2009; McEwen, 1998).

Physical stressors can include starvation (Romero & Wikelski, 2010), heat, noise, fire, working conditions, and pollution (Soewondo, 1996). Physical activity is another type of stress (i.e., physiological) that involves an almost identical initial physiological response to the aforementioned psychological stressors (e.g., increased heart and respiration rates, etc.), with the one exception being vessel response (e.g., vasodilation/vasoconstriction) which may depend on the type of receptor activated (Stipanuk, 2006a). Though the acute response is very similar to that resulting from psychological stress, the acute and chronic benefits to exercise are extensive. Several benefits of exercise interventions have been documented such as improved endothelial

functioning (Beck, Martin, Casey, & Braith, 2013), glucose transporter-4 (GLUT-4) translocation (Richter & Hargreaves, 2013) (i.e., translocation of the GLUT-4 receptor to allow for improved cellular glucose uptake), improved muscular strength, bone development, and antioxidant profile associated with decreased markers of lipid and protein oxidation (Chen, 1995; Finaud, Lac, & Filaire, 2006; Gomez-Cabrera, Domenech, & Vina, 2008). It is important to note however that the combination of psychological and physiological stress can pose many threats to an individual's health. A common theme is that *moderate* amounts of stress are needed for optimal performance. This concept does not only apply to psychological stress and performance (Benson & Allen, 1980), but physical as well since moderate amounts of physical stress are needed for several beneficial adaptations (Chen, 1995). Excessive exercise results in oxidative stress (Powers & Jackson, 2008) which could chronically contribute to down-regulated antioxidant status as well as other potentially detrimental health issues. In addition, the implications in terms of health risks related to dual stress challenges are significant since the exacerbated production of various markers of stress can increase an individual's risk for developing CVD (Huang, Webb et al., 2013; Webb et al., 2008; Webb, Garten et al., 2011; Webb et al., 2013). The potential mechanisms correlating stress and CVD risk will later be discussed in greater detail.

A thorough understanding of physiology of the stress response requires an understanding of the origin. The stress response, also known as the fight or flight response (Bers & Despa, 2009; Fuller, Emrick, Sadilek, Scheuer, & Catterall, 2010) serves the purpose of preparing the body to defend against, or flee from the scene of a life threatening stressor. Each acute physiological adaptation from this response has a direct

and distinct purpose, with the main purpose of this response being survival (Curtis & O'Keefe, 2002; Romero & Wikelski, 2010). Cortisol, glucagon, and catecholamines are referred to as the stress hormones since stresses of any type cause an increase in their circulatory concentration (Stipanuk, 2006a). The most significant cardiovascular and pulmonary responses occur via actions of two classes of hormones: adrenergic catecholamines and glucocorticoids. Catecholamines are adrenergic hormones secreted from the adrenal medulla (Stipanuk, 2006a) and are referred to as epinephrine and norepinephrine or adrenaline and noradrenalin, respectively. Epinephrine is released into the blood stream at the onset of a stressor, or during periods of starvation; norepinephrine's activity is stimulated via nerve endings directly at tissue for a localized effect (Stipanuk, 2006a). Catecholamines activate β-adrenergic receptors which activates adenylyl cyclase (Bers & Despa, 2009; Fuller et al., 2010). Elevations of cyclic adenosine monophosphate (AMP) concentrations are accompanied with phosphorylation by adenosine 3′,5′-monophosphate–dependent protein kinase which contributes to increased respiration rate and skeletal and cardiac tissue contraction via increased L-type Ca^{2+} activity (Bers & Despa, 2009; Fuller et al., 2010). The action of catecholamines depends largely on adrenergic receptor concentrations. Two α (α1 and α2), and three types of β-receptors exist (Frayn, 2003; Lafontan & Berlan, 1993). The β-receptors are associated with lipolysis, glycogenolysis, and gluconeogenesis. The α1 receptor is involved in glycogenolysis, while α2 receptors function to inhibit lipolysis (Stipanuk, 2006a). During periods of caloric restriction, intra-abdominal lipid stores tend to be most utilized in terms of energy production, compared to subcutaneous lipid stores which could be related to the receptor concentration. Subcutaneous adipose tissue tends to have greater concentrations

of α2 receptors, while intra-abdominal adipose tissue tends to have greater β-receptor concentrations. Regardless of the miniscule differences, during periods of stress or starvation one of the main functions of these hormones is to stimulate lipid and glucose mobilization. In addition, activation of α-adrenergic receptors results in an acute vasoconstrictive response, while β-adrenergic receptor activation induces vasodilation (Stipanuk, 2006a), increased respiration rate and contractility of skeletal and cardiac tissue via increased L-type Ca^{2+} activity (Bers & Despa, 2009; Fuller et al., 2010).

Stress and Cardiovascular Disease

Though this response is meant for survival purposes, chronic and/or improper sympathetic activation can potentially lead to an increased risk for the development of serious health issues including (but not limited to) reproductive disorders (Francis, 1981; Retana-Marquez, Bonilla-Jaime, Vazquez-Palacios, Martinez-Garcia, & Velazquez-Moctezuma, 2003), hypertension (Julius & Majahalme, 2000), metabolic syndrome (Chandola et al., 2006), CVD (Huang, Webb et al., 2013), inhibited bone development (Weinstein, Jilka, Parfitt, & Manolagas, 1998), as well as impaired memory and mental function (e.g., anxiety and depression) (Esch, Stefano, Fricchione, & Benson, 2002). In relation to CVD, chronic sympathetic activation is also involved in the progression of atherosclerosis (Huang, Webb et al., 2013) as well as impaired endothelial functioning, increased cardiovascular workload, left-ventricular hypertrophy, dysrhythmias (Metra et al., 2000) and oxidative stress (Huang, Wang, Liu, Zheng, & Li, 2013). Insulin resistance is also associated with chronic stress which is not only problematic in terms of improper glucose metabolism (i.e., diabetes progression) but can also contribute to elevated CVD risk (Curtis & O'Keefe, 2002). Elevated fasting insulin levels are also associated with

increased heart rate and sympathetic activation (Anderson, Hoffman et al., 1991; Festa, D'Agostino, Hales, Mykkanen, & Haffner, 2000).

It is important to reiterate that acute effects of stress (i.e., increased heart rate, blood pressure, and impaired metabolic efficiency) (Crews & Landers, 1987; Hamer, Taylor, & Steptoe, 2006; Webb, Fabianke-Kadue et al., 2011; Webb, Garten et al., 2011) are potentially beneficial while chronic stress can lead to atherosclerosis, hypertension, and impaired metabolic functioning as outlined above. Of great relevance to the progression of atherosclerosis is the noted impairment of endothelial functioning (Singhal, 2005) commonly distinguished by inadequate flow mediated dilation in response to stress (Ghiadoni et al., 2000; Szijgyarto, King, Ku, Poitras, Gurd, & Pyke, 2013). These characteristics of impaired vascular functioning are notably more prevalent and severe in firefighters and law enforcement officers (Fahs et al., 2009; Violanti et al., 2006) as they are chronically under high stress (Huang, Webb et al., 2013). This evidence has been supported upon investigation of aortic and carotid artery functioning in firefighters (Fahs et al., 2009) as well as an acute response subsequent to firefighting activities (Fahs et al., 2011; Fahs et al., 2012). Several investigations have shown cortisol secretion to be higher in both firefighters and police officers at rest and in acute response to psychological stress (Robinson, Leach, Owen-Lynch, & Sunram-Lea, 2013; Rosati et al., 2011; Tomei et al., 2003) which has also been shown to be related to impaired endothelial functioning (Violanti et al., 2009). These findings demonstrate that over-activity of the HPA axis may be a potential mechanism related to the elevated prevalence of CVD seen in high-stressed occupations (Huang, Webb et al., 2013).

Chronic stress may also result in oxidative stress and hyperlipidemia which can serve as another mechanism for atherosclerosis (Devaki et al., 2013). Oxidative stress is known to result in oxidation of lipids and DNA such as low density lipoprotein (LDL) which can contribute directly to the development of atherosclerosis (Hinterwirth, Stubiger, Lindner, & Lammerhofer, 2013; Pastori, Carnevale, & Pignatelli, 2014). A major function of the stress response is to stimulate the SA and HPA axes, resulting in increases in epinephrine, norepinephrine, and cortisol. Considering that these hormones function to mobilize energy stores (e.g., lipids, glucose), the potential combined effect of oxidative stress and excessive HPA/SA activation as induced by chronic stress on the development of CVD and atherosclerotic progression is significant and inter-related.

Another action of corticoids is to activate histone deacylase (HDA) at the glucocorticoid receptor which can deacylase histones and inhibit transcription of inflammatory cytokines. However, oxidative stress can *inhibit* HDA, allowing for histone acylation and transcription of pro-inflammatory molecules subsequent to activation of nuclear factor κB (NF κB) resulting in inflammation (Durackova, 2010; Ren et al., 2013; Sack, 2002) which may contribute to additional oxidative stress. Considering that oxidative stress occurs as a result of both physiological and psychological (Moller, Wallin, & Knudsen, 1996) as well as the combination of the two (Huang, Webb, Evans et al., 2010; Huang, Webb, Garten et al., 2010), the potential implications for individuals that work in high stress environments are significant (Huang, Webb et al., 2013; Webb et al., 2010; Webb, Garten et al., 2011). However, Cortez-Cooper et al. (2013) reported that the receptor activity of these cytokines might serve as an alternative indicator of impaired endothelial function in relation to CVD risk. While acute activation of cytokines such as

tumor necrosis factor-alpha (TNF-α) has several physiological benefits, it should be noted that chronic TNF-α production is potentially dangerous from a cardiovascular and metabolic standpoint (Foster & Samman, 2012; Sack, 2002). Chronic inflammation is best described by increased levels of cytokines such as TNF-α, and interleukin (IL)-1β, IL-2, IL-6 (Foster & Samman, 2012). Some specific cytokines associated with impaired endothelial functioning and vascular inflammation include TNF-α and IL-6 (Ross, 1999). The role of TNF-α in endothelial dysfunction and atherosclerotic progression has been extensively investigated (Fragoso Lona, Sierra Martinez, Vargas Alarcon, Barrios Rodas, & Ramirez Bello, 2013; Moreau, Deane, Meditz, & Kohrt, 2013; Sack, 2002; von Hundelshausen & Weber, 2013). A recent review by Lu, Zhao, Zhang, and Jiang (2013) reported the well distinguished correlation between chronic stress, cytokine production and increased risk for CVD via atherosclerosis. In addition, inhibition of this cytokine has been shown to *improve* endothelial functioning (Moreau et al., 2013; von Hundelshausen & Weber, 2013). Increased levels of monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) as induced by TNF-α (Huang, Wang et al., Li, 2013; Yao et al., 2014) may serve as a mechanism of increased atherosclerotic risk (Mohammadpour et al., 2014). It is also interesting to note that LDL cholesterol deposition in vascular endothelial cells may actually contribute to additional TNF-α production (Napoli et al., 2000; Niemann-Jonsson, et al., 2000).

The magnitude of cytokine production resulting from psychological stress may vary based on the intensity and duration of the stressor (i.e., acute versus chronic) (Huang, Webb et al., 2013). Chronic or severe stress has been shown to result in

increased production of inflammatory markers as well as free radicals (Dunn & Koo, 2013; Jankord, Zhang, Flak, Solomon, Albertz, & Herman 2010; Lu et al., 2013). Since elevations of TNF-α and IL-6 levels in response to psychological stress have been reported (Heinz et al., 2003; Steptoe, Willemsen, Owen, Flower, & Mohamed-Ali, 2001), the implications for individuals working under high stress conditions are significant (Huang, Webb et al., 2013). Specific to this population was an investigation that reported elevated serum IL-2 levels in response to concurrent stress (i.e., psychological and physiological) compared to physical stress alone (Huang, Webb, Garten et al., 2010). Similar findings have been reported among police officers in response to a simulated workplace event (Groer et al., 2010). Cytokines serve as biomarkers of inflammation; however, it is unclear at this point as to whether or not interventions should address circulating cytokine production to reduce the potentially dangerous effects of chronic stress (Lu et al., 2013). The well documented exacerbated cortisol production in response to concurrent stress (Webb et al., 2008; Webb et al., 2010; Webb, Fabianke-Kadue et al., 2011; Webb, Garten et al., 2011; Webb et al., 2013) is likely a greater threat from chronic stress as opposed to cytokine production alone.

Carbohydrates

Structure/Origin

In terms of structure, all CHOs contain carbon, hydrogen, and oxygen, with several hydroxyl (OH) groups as well as a functional carbonyl (C=O) group which can be expressed as either an aldehyde or a ketone (Gropper & Smith, 2013). Carbon numbers in monosaccharides range from three to seven. Carbohydrates are a major source of energy for humans as glucose is an essential nutrient for some cells and in some cases such as

red blood cells (RBCs), glucose serves as the only option for energy (Stipanuk, 2006b). Glucose can be derived directly via oral sources such as starch, sucrose, and lactose, and can also be synthesized in vivo via gluconeogenesis or glycogenolysis (Stipanuk, 2006b). Carbohydrates from food sources represent approximately 32-70% of total dietary intake in American and Canadian populations (McGrane, 2006).

Carbohydrates can be classified into two categories: simple and complex (Gropper & Smith, 2013). Monosaccharides and disaccharides are considered simple CHOs. Monosaccharides are sugars that cannot be reduced via hydrolysis. The most abundant sugar in nature is the 6-carbon monosaccharide, glucose. Disaccharides contain two monosaccharides covalently bonded via glycosidic bonds. Olgiosaccharides are considered complex and consist of at least three monosaccharides in a chain. Olgiosaccharides can be referred to as tri, tetra, or pentasaccharides with the variable prefix indicating the number of monosaccharides in the chain. Polysaccharides are the most complex and can contain as many as a few hundred or thousand monosaccharides. The most significant polysaccharide in terms of nutrition and physical performance is glycogen (Gropper & Smith, 2013).

Humans and animals store CHO as glycogen, while plants store CHO as starch. Both are polysaccharides that contain α1-4 covalently bonded glucose molecules (Stipanuk, 2006b). Starches can exist in either linear or branched form, with amylose being the linear structure and amylopectin being the more branched structure and usually contains 20 to 25 glucose units between branch points (Stipanuk, 2006b). Glycogen contains a branched structure similar to amylopectin with α1-4 chains and α1-6 branches. The average chain length contains 10 to 14 glucose units between branches.

Transport/Metabolism

Glucose homeostasis is primarily maintained by the liver (McGrane, 2006). Glucose can be stored as glycogen, degraded for energy, or the carbon skeletons can be donated and used for in vivo synthesis of amino acids or fatty acids. Complete oxidation of glucose can occur in cardiac and skeletal muscle. The metabolic demands of these two tissues however are tremendously different, since the heart has a constant demand for energy, while skeletal muscles can have tremendous variability in their metabolic demand for energy. In adipose tissue, glucose can either be partially or completely oxidized. Partial degradation via glycolysis can provide glycerol for triacylglycerol (TAG) synthesis. Additionally, excessive CHO intake can result in the metabolism of glucose to acetyl-coA, which in the absence of energy demand, can act as a precursor for fatty acid synthesis and storage (McGrane, 2006). Glucose serves as the brain's main source of energy; however, ketone bodies can be used in periods of starvation (McGrane, 2006). The brain has a constant demand for energy but does not have the ability to store glucose, as glucose is stored in the liver and skeletal muscles.

Glucose uptake in muscles and adipose tissue is driven by insulin, while glucose uptake in the liver, brain, and RBC is insulin independent (McGrane, 2006). Five glucose transporters (GLUT 1-5) are involved in the facilitated transport process by which cellular glucose uptake occurs. GLUT-1 is present in RBCs and the brain, as well as placenta and fetal tissues. GLUT-2 is in the liver and the β-cells of the pancreas, as well as the small intestine and kidney. Glucose uptake via GLUT-2 can act as a signaling sensor of glucose levels and stimulate insulin secretion. GLUT-3 is in the brain and placenta as well as slow twitch fibers in adults. GLUT-4 is the insulin dependent

transporter and is located in skeletal muscle and adipose tissues and is involved in maintaining glucose homeostasis for the entire body (McGrane, 2006). Exercise has been shown to induce GLUT-4 translocation independent of insulin (Richter & Hargreaves, 2013). This effect may be enhanced when coupled with whey protein after exercise (Morato et al., 2013). Adenosine monophosphate protein kinase (AMPK), Ca^{2+}, and nitric oxide synthase (NOS) are important signaling molecules which are initiated via exercise induced muscle contraction that assists in the regulation of GLUT-4 expression (Richter & Hargreaves, 2013). These findings are especially important for the treatment and possible prevention of type 2 diabetes (Morato et al., 2013). GLUT-5 is the transporter for fructose and is most predominantly found in the intestine, kidney, brain and skeletal muscle as well as adipose tissue (Gropper & Smith, 2013).

The body's demand for energy determines the fate by which CHOs are metabolized (Gropper & Smith, 2013). The various pathways CHOs are metabolized are listed as follows: glycogenesis (generation of new glycogen), glycogenolysis (breakdown of glycogen), glycolysis (oxidation of glucose), gluconeogenesis (synthesis of new glucose from non-glucose precursors), pentose phosphate pathway (generation of 5-carbon monosaccharides and nicotinamide adenine dinucleotide phosphate (NADPH), and the tricarboxylic acid (TCA) cycle, also known as Krebs' cycle, which is best described as the oxidation of acetyl-coA to form carbon dioxide and water (Gropper & Smith, 2013). Glycolysis will be the main source of discussion since this is a very significant ATP pathway that is heavily stressed during both high intensity exercise (Ziemann et al., 2011) and in the fight or flight stress induced scenario (Atkinson & Milsum, 1983). Recent studies also suggest that pro-inflammatory cytokines such as

TNF-α (Vaughan, Garcia-Smith, Dorsey et al., 2013; Vaughan, Garcia-Smith, Trujillo, & Bisoffi, 2013) can inhibit mitochondrial ATP production (driven by oxidative phosphorylation) and increase glycolytic ATP production. This is a phenomenon known as Warburg metabolism which may promote cancer cell proliferation (Cordero-Espinoza & Hagen, 2013; James, Chan, Erice, Siriwardena, & Bruce, 2013).

Glycolysis is broadly described as the progressive degradation of 6-carbon glucose monomers into two 3-carbon pyruvates, and the generation of ATP via substrate level phosphorylation (Brooks, Fahey & Baldwin, 2005). Two highly regulated steps of glycolysis are driven by hexokinase (muscle-bound) and/or glucokinase (liver-bound), as well as phosphofructokinase (Gropper & Smith, 2013). Each of these enzymes are inhibited by increased concentrations of their byproducts (Khitan & Kim, 2013). The subsequent metabolic fate of pyruvate is determined based on factors such as cellular oxygen availability and the body's demand for energy. Low cellular oxygen concentrations favor the conversion of pyruvate to lactate via lactate dehydrogenase (LDH) (a conversion that is not optimal during exercise since excessive lactate accumulation is associated with fatigue) (Jimenez-Reyes, Molina-Reina, Gonzalez-Hernandez, & Gonzalez-Badillo, 2013). LDH may also be regulated by proliferator-activated receptor-γ coactivator 1α (PGC-1α) (Summermatter, Santos, Perez-Schindler, & Handschin, 2013) which is a signaling molecule also associated with mitochondrial biogenesis (Davinelli, Sapere, Visentin, Zella, & Scapagnini, 2013). Exercise induced up regulation of PGC-1α expression can tremendously improve exercise performance (Summermatter, Santos, Perez-Schindler, & Handschin, 2013) by increasing the ability to oxidize lipids as energy, while decreasing dependency on CHO. This adaptation can also

be induced by treatment with polyphenols (i.e., resveratrol and equol) (Davinelli et al., 2013). Inhibited pyruvate dehydrogenase complex can also affect the rate by which pyruvate is converted to acetyl-coA (Sugden & Holness, 2003).

Carbohydrates in Relation to Health

In terms of dietary CHO promoting health, recent studies have shown that high dietary whole grain consumption can reduce the risk for developing type 2 diabetes (Aune, Norat, Romundstad, & Vatten, 2013) and CVD (Khosravi-Boroujeni et al., 2013; McKeown, Meigs, Liu, Wilson, & Jacques, 2002) while high intakes of white rice might increase chances of developing type 2 diabetes (McKeown et al., 2002). Increased consumption of fruits, vegetables, and whole grains might also decrease risk for multimorbidity (Ruel et al., 2013) which is described as an individual having the presence of at least two medical issues (van den Akker, Buntinx, & Knottnerus, 1996) and may lead to the development of at least one chronic disease (Ruel et al., 2013). This finding (Ruel et al., 2013) may be related to the elevated concentrations of phytochemicals, dietary fibers, iron, vitamin C, and magnesium in whole grains. Also reported was that white rice consumption was generally lower in healthy individuals while whole grain consumption was higher. This may be related to the elevated phytochemical content of whole grain, as well as the lack of dietary fiber in refined rice (Dixit, Azar, Gardner, & Palaniappan, 2011). Other studies reported that elevated white rice consumption may serve as a risk factor for developing hyperglycemia (Shi, Taylor, Hu, Gill, & Wittert, 2012) and type 2 diabetes (Nanri et al., 2010). This could be related to the fact that the refining process of white rice is such that the outer layer of the grain that contains the bran and germ is separated (Radhika, Van Dam, Sudha, Ganesan, &

Mohan, 2009). This process also removes minerals, phytoestrogens, vitamins, and phenolic compounds that may prevent the development of CVD and diabetes (Slavin, 2003). White rice consumption was further investigated by another recent study (Khosravi-Boroujeni et al., 2013) which failed to find a positive correlation between white rice consumption and CVD risk factors. However, this study used a food frequency questionnaire which may lead to inaccurate reporting of grain intakes (Hu et al., 1999). Several studies have documented negative effects associated with refined grain consumption, ranging from elevated fasting glucose levels (Sahyoun, Jacques, Zhang, Juan, & McKeown, 2006), hyperinsulinemia (Wirfalt et al., 2001), stroke (Liang, Lee, & Binns, 2010), and hypertriglyceridemia (Esmaillzadeh, Mirmiran, & Azizi, 2005). There have been contradictory reports regarding health risks and refined grain intake (Newby et al., 2007) which may be related to the variation in digestion and absorption rates of various grains (Khosravi-Boroujeni et al., 2013).

Dietary fructose ingestion is another area that has been gaining increasing interest. Fructose is in fruits and honey as well as table sugar (sucrose) which contains fructose and glucose. High fructose corn syrup is a food additive used for additional sweetness. High fructose corn syrup contains a mixture of fructose and glucose of varying concentrations (Khitan & Kim, 2013). Glucose and fructose are similar in structure but are metabolized differently, and they utilize different transporters (Zhao & Keating, 2007). Fructose metabolism is unregulated as it bypasses the highly regulated steps of glycolysis controlled by glucokinase/hexokinase and phosphofructokinase. These enzymes are inhibited by increasing concentrations of byproducts. Fructose instead initially enters glycolysis via fructokinase or ketohexokinase and is converted to fructose-

1-phosphate which has no negative feedback system (Khitan & Kim, 2013). In turn, excessive fructose metabolism results in detrimental cellular events such as phosphate depletion, activation of AMP deaminase, and excessive uric acid production (Fox & Kelley, 1972; Johnson et al., 2009; Maenpaa, Raivio, & Kekomaki, 1968). Uric acid is known to inhibit endothelial functioning (Khosla et al., 2005), raise blood pressure, activate the renin-angiotensin system (Feig et al., 2006) as well as impair adipocyte function (Sautin, Nakagawa, Zharikov, & Johnson, 2007). In addition, excessive fructose metabolism leads to hypertriglyceridemia which can increase visceral adipose deposition (Khitan & Kim, 2013) and is associated with activation of protein kinase C and hepatic insulin resistance (Stanhope & Havel, 2008).

Fructose also plays a direct role in lipogenesis since unregulated metabolism results in excessive pyruvate formation (from glycolysis) which is converted to acetyl-coA via pyruvate dehydrogenase. Acetyl-coA can bind with oxaloacetate to form citrate and will initiate Krebs' cycle if oxygen is present and there is a demand for additional energy production. However, in a fed state with low energy demands, the citrate can be transported to the cytosol to donate the coA used for lipid synthesis (Khitan & Kim, 2013). Fructose from natural sources such as fruits and 100% fruit juice may be less harmful since they contain additional nutrients and antioxidants (Khitan & Kim, 2013). Some evidence suggests that 100% fruit juice consumption might decrease the likelihood for developing insulin resistance (Pereira & Fulgoni, 2010).

Chronic and excessive high fructose or glucose intake has also been associated with oxidative stress as quantified by increased NAD(P)H oxidase activity (Shen, 2010). The subsequent result is potential adipocyte dysfunction and additional adipocyte

deposition in the muscle, liver, and pancreas (Khitan & Kim, 2013). Adipose tissue plays an important role in vascular function, lipid metabolism, as well as insulin regulation. Impaired vascular function as induced by excessive glucose or fructose intake is also associated with elevated cytokine production such as TNF-α and IL-6, as well as a decrease in adiponectin activity which can have an adverse effect on pancreatic β cells (Khitan & Kim, 2013). This finding is associated with enlargement of adipocytes which causes unfavorable changes in secretion of adipokines (Khitan & Kim, 2013).

To summarize, an overwhelming amount of research has documented adverse metabolic effects associated with high dietary fructose intake (Khitan & Kim, 2013). A discrepancy in findings exists between risk for CVD and white rice intake (Khosravi-Boroujeni et al., 2013). However, fructose is a potentially beneficial addition to athletic beverages since the combination of fructose with glucose allows for the use of different transporters and enhanced absorption (Jeukendrup, 2004).

Carbohydrates in Relation to Stress/Metabolism

The effects of CHO intake on exercise performance has been extensively evaluated and shown to be ergogenic (Smith et al., 2010; Sugiura & Kobayashi, 1998). Of greater relevance to this project is the potential ability of CHO intake to have an effect on the production of various inflammatory markers in response to both mental and physical stress. Recent investigations have shown that when these two stressors are combined, the cortisol response is exacerbated (Webb et al., 2008; Webb, Fabianke-Kadue et al., 2011). Chronic activation of stress hormones such as cortisol and other markers of inflammation may impair vascular functioning and increase platelet aggregation (Webb, Garten et al., 2011), and may serve as a potential mechanism for the

increased prevalence of CVD among active duty firefighters (Kales et al., 2003; Kales et al., 2007; Melius, 2001; Smith et al., 2012). It is important to address that a higher level of cardiovascular fitness may serve to better prepare an individual for dual stress challenges (Acevedo et al., 2006; Webb et al., 2013). However, several investigations have reported that CHO intake can improve cardiovascular responses to both psychological (Sihvola et al., 2013) and physical stress (Anderson, Bryden et al., 1991; McAnulty et al., 2003; Nehlsen-Cannarella et al., 1997; Nieman et al., 1998; Nieman, 1998). It has also been reported that high CHO diets may allow for some level of protection against oxidative stress (Moller et al+ 1996), which has been shown to result from dual stress challenges (Webb, Garten et al., 2011).

The relationship between cortisol and glucose levels has been investigated (Hucklebridge, Clow, Abeyguneratne, Huezo-Diaz, & Evans, 1999; Stull & Rodiek, 1988). One major role of cortisol is to maintain glucose levels (Friedmann, Goodman, & Weinhouse, 1967) and mobilize energy stores at the onset of a stressor (Kirschbaum et al., 1997), which may be achieved via gluconeogenesis and/or glycogenolysis. In a short term fasted state (such as an overnight fast), glycogen levels may be depleted which increases activation of gluconeogenic pathways (Landau et al., 1996). Several reports have shown that cortisol levels tend to peak in the morning upon waking (Hucklebridge et al., 1999) which may be related to a gluconeogenic role of cortisol (Friedmann et al., 1967; Khani & Tayek, 2001; Plas & Nunez, 1976). Although this response is quite universal, a small number of individuals (approximately 10%) fail to demonstrate this morning spike in cortisol production (Pruessner et al., 1997). However, data from Hucklebridge et al. (1999) suggest that low glucose levels upon awakening are not

necessarily correlated with exacerbated cortisol production. This finding indicates that the awakening cortisol response may be independent of glucose levels and not related to the stress induced gluconeogenic cortisol response (Hucklebridge et al., 1999). However, further research is needed to confirm this hypothesis. In addition, it has been shown that CHO intake in the morning can attenuate the morning cortisol spike (Follenius, Brandenberger, & Hietter, 1982). Other studies have reported that a decrease in glucose levels during physical stress (i.e., exercise) stimulates the HPA axis to secrete adrenocorticotrophic hormone (ACTH) and cortisol (Tabata, Atomi, & Miyashita, 1984; Tabata, Atomi, Mutoh, & Miyashita, 1990; Tabata, Ogita, Miyachi, & Shibayama, 1991). It may be possible to prevent this response if an exogenous source of glucose is provided prior to the onset of stressor (Kirschbaum et al., 1997).

Carbohydrate ingestion has also been shown to be beneficial in terms of cortisol attenuation when ingested prior to, during, and/or after physiological stress (i.e., exercise) (Anderson, Bryden et al., 1991; de Oliveira Quirino et al., 2012; McAnulty et al., 2003; Nehlsen-Cannarella et al., 1997; Nieman et al., 1998; Nieman, 1998). Previous studies reported that prolonged low-intensity physical exercise stimulates the HPA axis and induces ACTH and cortisol secretion (Tabata et al., 1984; Tabata et al., 1990; Tabata et al., 1991). Additionally, Tabata et al. (1991) reported that low plasma glucose levels during exercise (below 3.3mM) elicits a significant rise in corticotropin-releasing factor (CRF) which contributes to significantly higher cortisol levels in response to physical stress than compared with individuals who ingested an exogenous source of glucose prior to exercise (Tabata et al., 1991). This is not surprising especially considering that starvation is a physiological stressor (Romero & Wikelski, 2010) and therefore low

glucose levels seen during starvation (as well as exercise) are likely to stimulate HPA responses.

It has also been shown that glucose infusion can also attenuate this response (Tabata et al., 1991) further indicating that low glucose levels, or improperly maintained glucose levels may contribute to higher ACTH and cortisol (Galbo, Holst, & Christensen, 1979) via activation of CRF (Tabata et al., 1984). Glucose infusion has also been shown to result in significantly lower *postexercise* ACTH and cortisol levels (Tabata et al., 1991). In addition, pre-exercise glucose infusion has been shown to significantly decrease heart rate responses to physical stress (Tabata et al., 1991). These researchers (Tabata et al., 1991) chose intravenous method of glucose infusion based on the suggestion that this method tends to target the brain's uptake of glucose (Oomura, Ooyama, Sugimori, Nakamura, & Yamada, 1974), whereas oral glucose ingestion tends to induce high glucose concentrations in the gastrointestinal tract which will likely result in elevated liver uptake (Niijima, 1982). However, several studies have shown oral glucose ingestion to be an effective means to reduce cortisol stress responses to stress (Davison & Gleeson, 2005; Follenius et al., 1982; Lancaster, Jentjens, Moseley, Jeukendrup, & Gleeson, 2003). It is also important to note that oral glucose ingestion, as opposed to intravenous, may be *more* effective at reducing other markers of inflammation such as IL-6 (Manning, Sutherland, Williams, de Jong, & Hendry, 2013) which may be related to increased insulin production (Elrick, Stimmler, Hlad, & Arai, 1964) and thus, greater glucose clearance rate following oral glucose ingestion as opposed to intravenous (Scow & Cornfield, 1954).

Decreased IL-6 and plasma peroxide concentrations following CHO ingestion (75g glucose) has also been reported (Manning, Sutherland, Walker, de Jong, & Berry, 2008). This finding provides support to the anti-inflammatory effect of insulin (Dandona et al., 2001; Manning et al., 2008). Carbohydrate ingestion also has the ability to increase tryptophan levels (Silber & Schmitt, 2010) which is significant since insulin facilitates the transport of tryptophan to cross the blood-brain barrier, thus allowing for subsequent synthesis of 5-hydroxytryptamine (5-HTP) (Le Floc'h, Otten, & Merlot, 2011; Sihvola et al., 2013). In addition to 5-HTP synthesis, glucose is the primary source of energy for the brain (Sihvola et al., 2013), as well as an immediate source of energy for active tissues during stress (Gilsenan, de Bruin, & Dye, 2009; Hoyland, Lawton, & Dye, 2008). An inverse relationship between CHO intake and cortisol levels has not only been noted after acute ingestion of CHO, but also after several days on a high CHO diet (Anderson et al., 1987). It is important to note that Anderson et al. (1987) also reported a reciprocal relationship between cortisol and testosterone, which further reinforces the role of cortisol as a catabolic hormone that opposes anabolic responses. Insulin is another anabolic hormone that is *inhibited* during stress via glucocorticoids (i.e., cortisol) (Chrousos, 2000), which is important to note since this suggests that the aforementioned findings (Manning et al., 2008) may not be applicable to stress induced inflammatory responses.

Timing of pre-exercise CHO ingestion prior to physical stress was investigated by Lancaster et al. (2003). A 75-gram dose of glucose was ingested at either 15 or 75 min prior to exercise. No significant differences were reported in terms of mean heart rate, mean power output, and time to completion between the two conditions. Ingestion of

glucose 75 min prior to the start of exercise resulted in a decrease of plasma glucose levels (below the pre-CHO ingestion levels). However, there was no difference between the two timing trials at the end of exercise. In addition, the ingestion of CHO 75 min prior to exercise resulted in significantly higher cortisol levels post exercise, whereas ingestion 15 min prior to exercise did not (Lancaster et al., 2003). These findings indicate that the timing of CHO ingestion has an effect on cortisol stress responses. Also investigated in this study (Lancaster et al., 2003) was the effect of the amount of CHO ingested on markers of performance and neutrophil/lymphocyte (N/L) ratio responses to exercise. No significant differences were reported in heart rate, mean power, or time to complete trial between individuals who consumed either 25 or 200g CHO as a glucose solution 45 min prior to exercise (Lancaster et al., 2003). Additionally, the amount of CHO ingested had no effect on N/L ratios, which is a marker of physiological stress and has been shown to be correlated with cortisol production (Nieman et al., 1999). Since the amount of CHO ingestion did not have an effect on N/L ratios, these researchers (Lancaster et al., 2003) hypothesized that there was likely no effect of varying CHO doses (25 versus 200g) on cortisol production as well since the N/L ratio has been shown to correlate with cortisol (Nieman et al., 1999). However, this suggestion is mere speculation since cortisol was not measured subsequent to the ingestion of varying amounts of CHO prior to exercise (Lancaster et al., 2003).

Carbohydrate ingestion has also been shown to attenuate cortisol subsequent to *prolonged exercise* (2.5 hours at 60% VO_2max) (Davison & Gleeson, 2005). This study (Davison & Gleeson, 2005) also incorporated a vitamin C supplement that was tested in combination with and separate from a CHO supplement. The findings showed that CHO

ingestion prevented a significant decrease in glucose levels and decreased post exercise cortisol and ACTH values when ingested both alone and with a vitamin C supplement. Also reported was a non-significant trend for consistently lower plasma IL-6 concentrations. In addition, a significantly lower measure of leukocytosis and neutrophilia post exercise was reported with both the CHO condition and vitamin C and CHO combined condition. Carbohydrate intake also induced a significant attenuation in the post exercise decrease of stimulated neutrophil degranulation (a measure of functional capacity) (Davison & Gleeson, 2005). This finding is significant since CHO ingestion may blunt the acute suppression of immune function seen from prolonged exercise which would increase susceptibility to infection (Nieman, 1997; Pedersen et al., 1999), and can potentially have an adverse effect on performance (Gleeson, 2000; Gleeson et al., 2000).

Although the majority of research has investigated the effects of CHO intake and physical stress (Anderson, Bryden et al., 1991; McAnulty et al., 2003; Nehlsen-Cannarella et al., 1997; Nieman et al., 1998; Nieman, 1998), it is also important to note that beneficial cardiovascular effects of CHO intake prior to mental challenges have been reported (Sihvola et al., 2013). This may include decreased heart rate response to a stressor, as well as improved mental coping and higher plasma tryptophan to other large neural amino acid ratios subsequent to high CHO intake (Sihvola et al., 2013). However, the efficacy for this nutritional intervention has not yet been established under dual stress conditions, which is necessary especially to provide a potential solution for the exacerbated cortisol responses resulting from these conditions (Webb et al., 2008; Webb, Fabianke-Kadue et al., 2011; Webb, Garten et al., 2011). Although several studies have investigated the effects of CHO intake on stress responses, many have failed to address

other markers of physiological stress such as catecholamines (Lancaster et al., 2003; Sihvola et al., 2013) which also play a role in glucose mobilization. It is imperative to expand upon previous findings and test the effects of CHO intake under dual stress conditions to provide potential implications for firefighters and other personnel that chronically experience such stress (Kales et al., 2003; Kales et al., 2007; Melius, 2001; Smith et al., 2012).

CHAPTER III

MATERIALS AND METHODS

IRB Approval

This project was approved by the Institute of Biosafety Committee (IBC) at Mississippi State University in October 2014, as well as the Institutional Review Board (IRB) at Mississippi State University in November 2014. All research personnel assisting with data collection were approved by the IRB. All personnel assisting with handling of human blood samples were approved by the IBC.

Participants and Study Design

Sixteen apparently healthy non-smoking males aged 21-30 years participated in this study. A standard screening procedure was followed that required participants to be low risk for CVD according to American College of Sports Medicine (ACSM) criteria (ACSM, 2014) as well as free of: (1) cardiorespiratory and/or metabolic disorders, (2) any known blood disorders (e.g., anemia, hemophilia), (3) hearing or vision disorders, (4) psychological/mental health disorders, (5) any use of prescription medication or tobacco products, and (6) any severe psychological trauma or significant stressor within 30 days of the study (e.g., divorce, death in family). Participants were unpaid native English speaking volunteers and they had to agree to follow the procedures outlined in the project,

A randomized, double blind, placebo controlled, cross-over design was used. Participants were tested on four separate occasions. During the initial visit, they provided written informed consent, completed a health and medical history report, and general procedures for testing were explained to participants. During session two, anthropometric data for height, weight, and waist circumference were obtained. Each participant also performed a VO_2 peak test, as well as a 90 sec familiarization session with the mental stress challenges. VO_2 peak was tested using a Velotron Racermate™ (Racer-Mate Inc., Seattle, Washington, USA) with initial workload starting at 100W. The initial workload was 100W for a five min stage. Each subsequent stage was three min in duration and corresponded with an increase by 50W until min 14. At min 14, workload was increased by 25W every min until volitional fatigue. Alternatively, the test was terminated if a plateau of VO_2 consumption was noted (<150ml/min) with increasing workload, or in the presence of two of the following: (1) reaching age-predicted maximum heart rate, (2) respiratory exchange ratio (RER) greater than 1.15, (3) RPE value greater than18 on a 15 point Borg Scale.

During the third and fourth visits, either a 6.6% CHO solution or non-CHO control beverage (flavored, non-caloric water) was randomly assigned in a cross-over manner and orally ingested at 0.6g/kg body weight 15 min prior to performing a dual concurrent stress (DCS) challenge. The 15 min ingestion of CHO solution was chosen since previous work by Lancaster et al. (2003) reported lower post exercise cortisol (CORT) production when CHO was ingested 15 min prior to exercise as opposed to 75 min prior. The supplement was a CHO-electrolyte product containing 6.6% CHO concentration that provided 110 kilocalories in 355 mL and the following ingredients:

water, sugar, dextrose, citric acid, potassium citrate, salt, sodium citrate, potassium phosphate, sodium phosphate, natural and artificial flavor, calcium phosphate, gum arabic, and artificial color. The placebo treatment contained zero calories and also included the following ingredients: water, citric acid, malic acid, gum arabic, sucralose (sweetener), natural and artifical flavor, acesulfame potassium (sweetener), sodium citrate, sucrose acetate, isobutyrate, sodium benzoate, and potassium sorbate. Participants were allowed to consume water ad libitum before and after exercise. Carbohydrate dosage was assigned at 0.6g/kg body mass. Since CHO sources were sucrose and dextrose, the involvement of glucose and fructose allows for the use of additional glucose transporters (Jeukendrup, 2004). Beverages were orally ingested 15 min prior to the onset of exercise, as similarly done by Lancaster et al. (2003). Participants were allowed five min to consume the beverage. Beverage flavor was similar between trials to avoid the possitibilty of palatability affecting psychological responses. Beverages were stored and served at 38 degrees F.

The DCS procedure consisted of a physical stress (i.e., exercise) combined with a mental stress. Exercise was performed on an electromagnetically braked cycle ergometer and workload was predicted based on steady state VO_2 values from the aforementioned peak protocol to estimate the workload corresponding with 60% VO_2 peak (Åstrand, Rodal, Dahl, Strømme, 2003). The mental stress challenge (MSC) consisted of a modified Stroop Color Word (SCW) and mental arithmetic (MA) which were performed on a computer mounted in front of the bike with a numeric and color-coded key pad mounted on the handlebars of the bike. The mounting placement was such that it allowed participants to maintain normal riding position with both hands placed comfortably on the

handlebars. Steady state exercise was performed for 35 min. The MSC was introduced 10 min into the exercise bout to allow participants to reach a physiological steady state, and lasted for a total of 20 min. General procedures are similar to Webb et al. (2013).

Testing Procedure

Participants reported to the J.A. Chromiak Applied Research Laboratory in the Department of Kinesiology at Mississippi State University on four separate occasions. During sessions three and four, participants arrived at the laboratory at 7:00am ± 21.5 min) in an 8-hour fasted state. Particpants were also required to refrain from strenuous physical activity and/or alcohol consumption for 24 hours prior to testing. A minimum of 48 hours of recovery was required between sessions two and three, while sessions three and four were separated by at least five days.

Upon arrival to the laboratory for sessions three and four, an intravenous catheter (B. Braun Introcan Safety, USA; 22 G, 25mm) was inserted into an antecubital vein. A 25cm minimum volume extension set was used (Tuta Healthcare, Lidcombe, NSW, Austrailia) which had a CLC2000 positive pressure connector (ICU Medical, Inc., San Clemente, California, USA) attached. Participants were then required to sit for 70 min at a quiet desk in an attempt to allow CORT to return to baseline levels since there was expected to be a response from the HPA axis associated with IV insertion. After 70 min, participants performed 35 min of steady state exercise at 60% VO_2 peak. All exercise conditions were performed on a Velotron Racermate™ (Racer-Mate Inc., Seattle, Washington, USA). Workload was controlled by Velotron Coaching Software (CS5) (Seattle, Washington, USA). The mental challenges were performed on a program designed by Macromedia Authorware (Version 5.0, Adobe Inc., Mountain View,

California, USA) specifically adjusted for consistent timing among data collection trials. The mental challenges began 10 min after the onset of exercise and were performed on a computer mounted in front of the bicycle for a total of 20 min. In addition, participants consumed either a 6.6% CHO solution or non-CHO control beverage (artificially sweetened water) which was randomly assigned and orally ingested at 0.6g/kg body weight 15 min prior to initation of exercise.

Instrumentation

A ParvoMedics TrueOne 2400 integrated metabolic measurement system (Parvo Medica, Sandy, Utah, USA) was used during each testing session to assess cardiorespiratory responses including the following: heart rate (HR), ventilation (V_E), volume of oxygen consumption (VO_2), volume of CO_2 expenditure (VCO_2), respiration rate (RR), and respiratory exchange ratio (RER). These responses were collected continuously during all exercise protocols. Air volume was measured using a Hans Rudolph 3813 pneumotachometer (Hans Rudolph, Inc., Kansas City, Missouri, USA).

Subjective perceived exertion was measured using the rating of perceived exertion (RPE) scale (Borg, Hassmen, & Lagerstrom, 1987). RPE was measured five min into exercise, as well as 10, 30, and 35 min into exercise. Psychological anxiety was measured with a short version of the State Anxiety Inventory (SAI) (Devito & Kubis, 1983) and recorded 70 and 40 min before the start of exercise, immediately at the onset of exercise, four times during exercise, as well as 15 min after exercise. Overall perceived demands of the workload were estimated with the NASA Task Load Index (NTLX) (Hart & Staveland, 1988) immediately after the participants dismounted the bike. Six subscales

were incorporated in this assessment and included mental demands, physical demands, temporal demands, performance, effort, and frustration.

Blood Sampling and Analysis

A total of 10 mL of blood was sampled at each blood draw. An initial aliquot of three mL of blood was drawn into a syringe and discarded. After each blood draw, a volume of physiological saline was administered to replace the volume of blood removed and was equal to half of the total amount of blood drawn. Blood was drawn into a sodium heparin vacutainer and centrifuged for 15 min at 1500 rpm and stored at -40 degrees C. Concentrations of CORT were measured with an enzyme linked immunozorbent assay (ELISA) (Pointe Scientific, Canton, Michigan, USA) using an iMark Bio-Rad microplate absorbance reader (Life Science Research, Hercules, California, USA). Glucose was measured using a glucose oxidase assay (Pointe Scientific, Canton, Michigan, USA) and a Pointe 180II spectrophotometer (Pointe Scientific, Canton, Michigan, USA). Changes in plasma volume for each of these timepoints were estimated by the method of Dill and Costill (1974). Potential changes in plasma volume over time were analyzed for blood draws corresponding with the following timepoints: 15 min prior to exercise, immediately at the onset of exercise, 10, 20, 30, and 35 min into exercise, as well as 15 min after exercise.

Blood was drawn at the following intervals: 70 min, 40 min, and 15 min prior to the start of exercise, immediately at the onset of exercise, as well as 10, 20, 30, and 35 min during exercise. In addition, blood was drawn at 15, 30, 45, and 60 min during a post exercise recovery period. During the pre and post exercise resting periods, the participants were seated on a chair at a desk in a quiet room with reading material

provided to them, which included popular magazines and scientific journals. CORT and glucose were measured for each of the 12 blood draw timepoints.

Mental Challenge

The mental challenges were performed on a computer mounted in front of the ergometer, with user controlled keypad mounted within comfortable reach, accommodating the dominant side of the participant. SCW and MA were performed using Macromedia Authorware software (Version 5.0, Adobe Inc., Mountain View, California, USA). For the SCW protocol, participants were presented with a 0.5 sec display of a word on the computer screen such as yellow, green, red, or blue. The word was written in a conflicting colored font and in addition, a third color was audibly presented within this 0.5 sec to instill mental conflict. Subsequent to the 0.5 sec display, the participants were presented with a blank screen for 0.5 sec. Participants were asked to recall the font color of the text that was displayed on the screen. Participants responded by using the color-coded keypad mounted on the handlebars of the bike. Feedback was given including total attempts, total correct and incorrect responses, as well as missed responses.

The MA challenge involved compupter based arithmatic calculations such as addition/subtraction with single, double, and triple digit numbers. The calculations are designed to be simple however when a participant reports an incorrect answer, an adverse audible was presented (i.e., buzzer or horn). The participant was allotted 10 sec to respond to each mathematical question. Failing to respond within this time resulted in an incorrect response.

Statistical Analysis

Statistical procedures were conducted using SAS 9.3 (SAS Institute, Inc. 2011, Cary, North Carolina, USA). Regarding the cardiorespiratory variables, changes in RER were determined by calculating the mean RER from 10 to 30 min during the exercise protocol since this was the dual stress exposure period. Differences in mean RER values between treatments were determined by paired t-tests. In adddition, to ensure consistency in workload, mean VO_2 values were calculated from 10 to 30 min during the dual stress period and paired t-tests were used to ensure that there were no significant changes in mean VO_2 between treatments. A 2 x 12 RMANOVA was used to investigate potential changes in CORT and glucose across time as well as between treatments. Tukey-Kramer's multiple comparison test was conducted in the instance of a significant main effect ($p < 0.05$).

Overall release of CORT was analyzed via integrated area under curve (AUC) calculation (Jones, 1997) and included all data from the onset of DCS through the post exercise recovery period. Paired t-tests were used to investigate differences in AUC calculations between treatments. Changes in RPE values were assessed via 2 x 4 (condition x time) RMANOVA. SAI levels were assessed via 2 x 8 (condition x time) RMANOVA. In the instance of significant main effects ($p < 0.05$), Tukey-Kramer's multiple comparison method was used to further investgate differences. Paired t-tests were used to investigate potential differences in NTLX values between treatments.

CHAPTER IV

RESULTS AND DISCUSSION

Results

Sixteen presumably healthy adult males with a mean age of 23.5 yrs ± 2.6 fully completed all testing sessions. Descriptive characteristics of participants are shown in Table 4.1 and a descriptive timeline of testing is presented in Figure 4.1. Regarding the two testing sessions that included the DCS, workload was the same as the mean VO_2 and was not significantly different between conditions (CHO = 2.02; PLA = 2.03; T = 0.60, p = 0.5568). The projected 60% workload was 2.05L*min^{-1}. The mean amount of CHO ingested was 50.5g ± 4.8. The mean fluid ingestion was 764mL ± 73. The mean workload during the sessions was 123.3 ± 26W. Mean RER values were calculated from 10 to 30 min during the exercise since this was the dual stress period (CHO = 0.93 ± 0.05, PLA = 0.90 ± 0.03). Carbohydrate ingestion resulted in a significant increase in RER values (T = 3.51, p = 0.0032).

Changes in SAI levels were not significantly different between treatments (F = 0.07, p = 0.785) but there was a significant main effect for time during exercise (F = 33.97, p < 0.0001) indicating that the dual concurrent stressor had a significant affect on SAI levels. SAI means are reported in Figure 4.2. There was a significant decrease in SAI from the end of exercise to 15 min post exercise recovery time point as determined by Tukey-Kramer multiple comparisons. In addition, SAI values were significantly higher

during exercise compared to when subjects were not exercising for both treatments. The highest SAI values were noted at the time point of 30 min which was immediately at the conclusion of the mental challenges. There was a nonsignificant decrease from time point 30 to time point 35 min which indicated that once the mental challenges subsided, the participants experienced a decrease in SAI.

There was not a significant interaction between treatments and time points for mean RPE levels during exercise ($F = 0.40$, $p = 0.744$) and there was not a significant difference between treatments in mean RPE ($F = 0.99$, $p = 0.321$). However, there was a significant main effect for time ($F = 18.92$, $p < 0.0001$) indicating that the dual concurrent stressor had a significant affect on perceived exertion. RPE means are demonstrated in Figure 4.3. There was no significant change in mean RPE from time point 5 to time point 10 min as indicated by Tukey-Kramer multiple comparisons. However, there was a significant increase from 10 to 30 min into exercise, which was likely attributed to the mental challenges. In addition, there was a significant reduction in RPE once the mental challenges concluded, despite no change in physical workload. The CHO supplement had no effect on the participants' perceived demands of the overall workload since there was no significant change in mean NTLX scores between treatments ($T = 1.41$, $p = 0.1786$).

There was a significant main effect for plasma volume changes over time ($F = 3.36$, $p = 0.0085$); however, Tukey-Kramer multiple comparisons showed no significant changes in plasma volume over time. The trend in mean CORT production over time is not significantly different between treatments and thus there was no significant treatment by time point interaction ($F = 0.18$, $p = 0.998$). There was a significant main effect for the

CHO and placebo treatments regarding mean CORT production ($F = 5.30$, $p = 0.0219$). A significant change in mean CORT was noted for at least two time points as well ($F = 10.31$, $p < 0.0001$). The highest CORT levels were noted immediately after IV insertion at 70 min before exercise. CORT levels were not significantly different at the initial blood draw upon IV insertion (CHO = 401.45 ± 79.81; PLA = 403.82 ± 60.74). There was a trend for decreasing CORT levels until the onset of exercise however, these reductions were not significant. For both treatments, there was a significant reduction in CORT levels from the end of exercise (35 min) to R60 (60 min after exercise). In addition, there was a significant reduction in CORT levels from R15 (15 min after exercise) to R60 for both treatments. The participants varied significantly in terms of their mean CORT production ($F = 12.14$, $p < 0.0001$). For the CHO treatment, the upper and lower 95% confidence levels for the CORT means were 349.9 and 325.0, respectively. In addition, for the PLA treatment the upper and lower 95% confidence levels for the CORT means were 361.1 and 332.6, respectively. This most likely explained the lack of significant differences among individual time points between treatments with post hoc analysis despite a significant main effect for treatment. In addition, one participant demonstrated CORT levels that were at least two standard deviations below the mean for time points 20, 30, 35, R15, R30 (30 min after exercise); however, such low values were only seen when this participant ingested CHO prior to testing. The mean values for CORT production for both treatments are demonstrated in Figure 4.4. For each time point, CORT levels tended to be lower during the CHO trial; however, the levels were not significantly different.

In an attempt to estimate the overall release of CORT production during both trials, AUC was calculated using the trapezoidal method (Jones, 1997) on the treatment means starting from the onset of the dual stressor (10 min into exercise) following through the 60 min recovery period. A paired t-test indicated that the total AUC for CORT was significantly less when CHO was ingested ($T_7 = 4.07$, $p = 0.0048$). This relationship can be interpreted as demonstrating a large effect size (Cohen's $d = 1.439$ (Cohen, 1988). In addition, the overall AUC was significantly less during the CHO trial from the start of exercise to the end of exercise ($T_4 = -3.35$, $p = 0.028$). Overall release of CORT was also significantly less during the 60 min post exercise recovery period ($T_4 = -3.34$, $p = 0.0289$) when CHO was ingested.

The mean glucose levels for both treatments are shown in Figure 4.5. The trend in mean plasma glucose levels over time were significantly different between the two treatments ($F = 10.92$, $p < 0.0001$) indicated by a significant treatment x time interaction. There was also a significant difference between the treatments in plasma glucose levels ($F = 13.04$, $p = 0.0004$), and at least two time points were significantly different with respect to mean glucose level ($F = 16.1$, $p < 0.0001$). There was no significant change in glucose levels before, during, or after exercise when PLA was ingested. Regarding the CHO trials, there was no significant change in glucose levels from 70 to 15 min prior to testing (when CHO was ingested). There was a significant increase from the time of CHO beverage consumption to the start of exercise (15 min later). There was no significant change in glucose levels from the start of exercise to 10 min into exercise. However, there was a significant decrease in glucose levels 20 min into exercise (10 min into the mental stress challenge). Glucose levels were significantly higher at time points 0 and 10

compared to all other time points. There were no other significant changes in glucose levels. The lowest mean glucose level was seen at time point 30 during the CHO trial (73.56 mg/dL ± 16.62). Six participants demonstrated glucose levels below 70 mg/dL at this time (four of them were below 60 mg/dL).

Discussion

Several studies have reported that CHO ingestion prior to physiological stress can affect subsequent CORT production (Anderson, Hoffman et al., 1991; McAnulty et al., 2003; Nehlsen-Cannarella et al., 1997; Nieman, 1998). However, to our knowledge this is the first study to investigate the effects of this acute nutritional intervention on CORT production to *combined* physical and mental stress. The main findings of this investigation are that CORT production to a DCS challenge is attenuated when CHO is ingested prior. There was a consistent nonsignificant trend for lower mean CORT production at each time point during and post exercise. In addition, overall release of CORT was shown to be significantly lower when CHO was ingested.

The DCS challenge was effective at increasing subjective anxiety as well as perceived exertion. It is important to note that there was a reduction in SAI after the mental challenges subsided, despite no reduction in physical workload. This indicated that the addition of the mental challenges to the physical workload was effective at increasing anxiety levels of the participants, which has been similarly shown in previous studies using MA and SCW to induce mental stress (Acevedo et al., 2006; Webb et al., 2013). Participants' anxiety levels significantly decreased immediately after exercise and nearly returned to baseline (pre-exercise levels) in a rapid fashion (i.e., within 15 min) which may be attributed to their regular participation in exercise (Hallgren, Moss &

Gastin, 2010). Further, the significant increase observed in RPE was likely due to one of two factors: (1) the duration of exercise, or (2) the combination of mental and physical stress. Since there was a significant decrease in RPE after the end of the mental challenges (despite no change in physical workload), the latter is most likely true. Hence, the DCS challenge resulted in a significant increase in perceived exertion that was not affected by CHO ingestion.

Timing of CHO ingestion was selected due to findings from Lancaster et al., (2003) with one main intention of avoiding reactive hypoglycemia during the onset of exercise. Low glucose levels were not seen before the onset of exercise. During the CHO trial, glucose concentrations significantly decreased from the start to the end of the DCS, returning near pre-ingestion levels, indicating that the participants were likely more dependent on the exogenous CHO during the DCS (Smith et al., 2010). These findings are supported by the significantly elevated RER values during the CHO trial. Our glucose data were similar to that from Lancaster et al. (2003) in that the highest means of glucose production were 0 and 10 min into exercise when glucose was ingested 15 min prior. In addition, glucose levels were unchanged during the PLA trial throughout exercise. Based on these findings, it appears as though glucose concentrations tend to remain elevated for 20 min into moderate intensity exercise when CHO is ingested 15 min prior. In addition, it also appeared that the decline in glucose levels during the mental challenges was likely not due to the combination of stressors.

The overall release of CORT was similar to the report from Webb et al., (2012) which showed that the combination of physical and mental stress results in excessive activation of the HPA axis. CORT is a glucocorticoid that functions to regulate glucose

metabolism. The hypothalamus contains glucose sensing receptors that can stimulate the release of ACTH, and subsequently CORT, if blood glucose concentrations fall during exercise (Tabata et al., 1991). The duration of exercise was not significant enough to elicit a drop in blood glucose concentrations in the absence of exogenous CHO. However, CORT production was still attenuated when exogenous CHO was provided. These findings were similar to a report from Lancaster et al. (2003) that observed lower CORT production when CHO was ingested 15 min prior to exercise as opposed to 75 min prior. However it is also apparent that reactive hypoglycemia may not be the main cause of excessive CORT production, but rather that the additional presence of exogenous CHO may reduce HPA activation via glucose sensitive receptors on the hypothalamus. Since hypoglycemia was not associated with increased CORT production, these findings also indicated that low plasma glucose concentrations during moderate intensity and moderate duration exercise were not necessarily a main cause of increased CORT production. However, one limitation to the present study was that catecholamine production to the DCS was not investigated. Catecholamines have been shown to increase in response to physical and mental stress and can also activate the anterior pituitary which may affect CORT production as well. This study was also limited by not including a mental stress alone condition or an exercise alone condition to investigate the effects of CHO ingestion and make comparisons between such treatments to further investigate treatment effects. However, previous studies have reported that CHO intake can improve cardiovascular responses to both psychological (Sihvola et al., 2013) and physical stress (Anderson, Hoffman et al., 1991; McAnulty et al., 2003; Nehlsen-Cannarella et al., 1997; Nieman, 1998). The purpose of this study was to investigate the effects of CHO ingestion on DCS

exposure with the intention of extrapolating findings to individuals chronically exposed to combined stressors.

Summary

To our knowledge this was the first study to investigate the effects of exogenous CHO ingestion on CORT production to dual concurrent stressors. Mean CORT production tended to be lower during and after DSC which serves as a potential beneficial implication for individuals that work in high stress occupations. Four out of 16 subjects had glucose levels that fell below 60 mg/dL at the end of the DCS. Therefore, future investigations should investigate the effects of *repeated* bouts of DCS and/or CHO ingestion and include an analysis on catecholamines and potentially biomarkers of oxidative stress. In addition, it is apparent that the DCS was effective at increasing anxiety and stress; however, the magnitude by which this can be compared to practical scenarios (e.g., burning building) for high stress occupations cannot be speculated. Future studies should also investigate the potential effect of CHO ingestion during such circumstances.

Table 4.1 Characteristics of participants.

Age (yrs)	Height (cm)	Weight (kg)	VO_2 Peak ($L*min^{-1}$)	Waist (cm)
23.5 ± 2.6[a] (21-30)	178 ± 7.1 (164.3-188.7)	83.1 ± 9.1 (69.5-97.9)	3.4 ± 0.5 (2.8-4.5)	84.7 ± 4.8 (75-91)

[a]Means ± standard deviations (ranges).

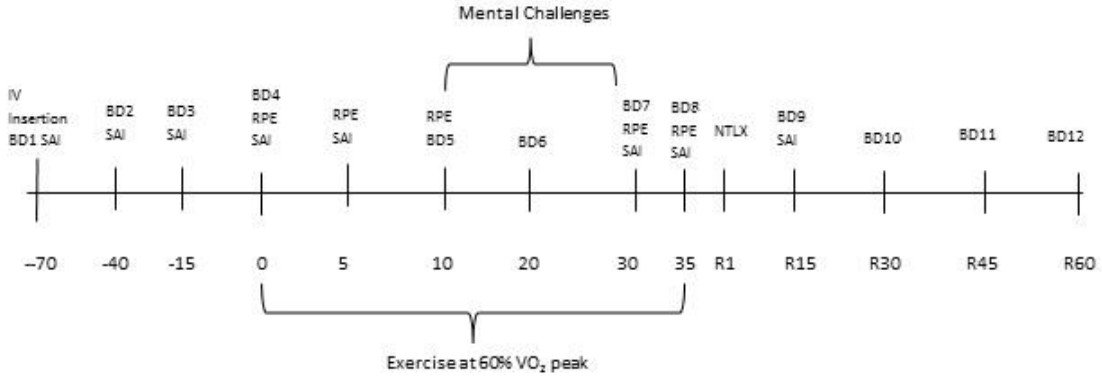

Figure 4.1 Timeline of testing events, adopted from Webb et al. (2013).

BD = blood draw. R = post exercise recovery period. SAI = State anxiety inventory. NTLX = NASA task load index. RPE = Rating of perceived exertion.

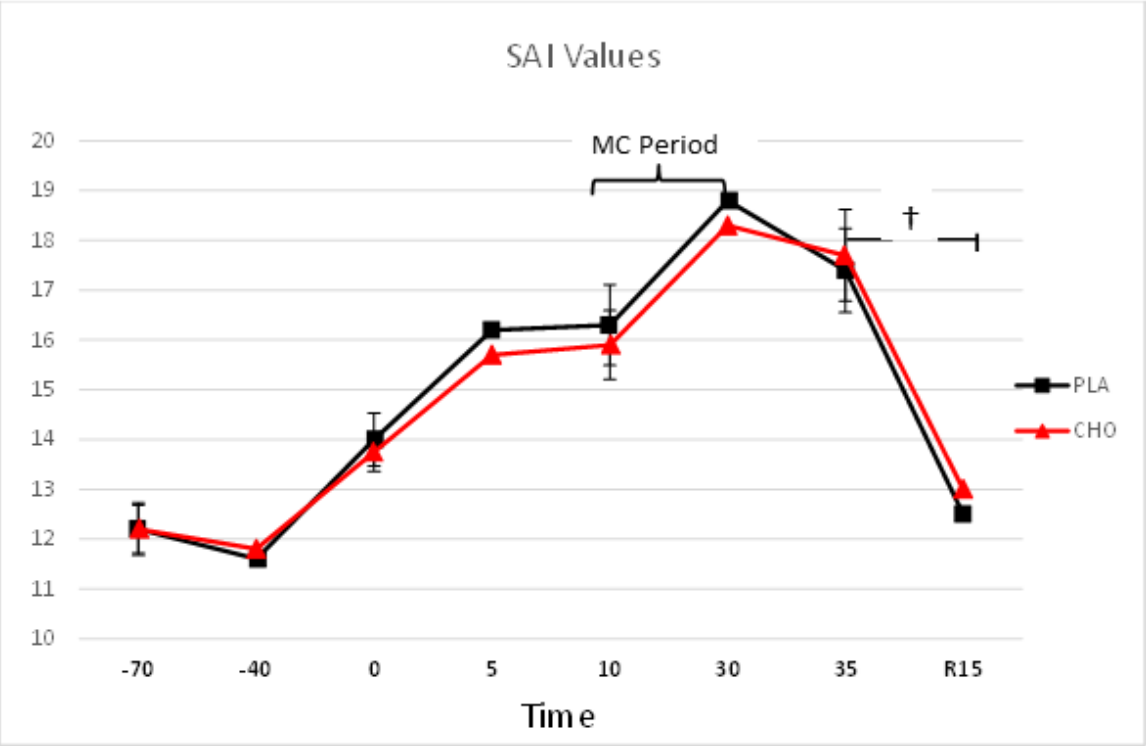

Figure 4.2 Changes in State Anxiety Inventory (SAI) over time.

PLA = placebo condition; CHO = carbohydrate condition. MC = mental challenge. -70 = 70 min before exercise. 0 = start of exercise. 35 = end of exercise. R = post exercise recovery period.

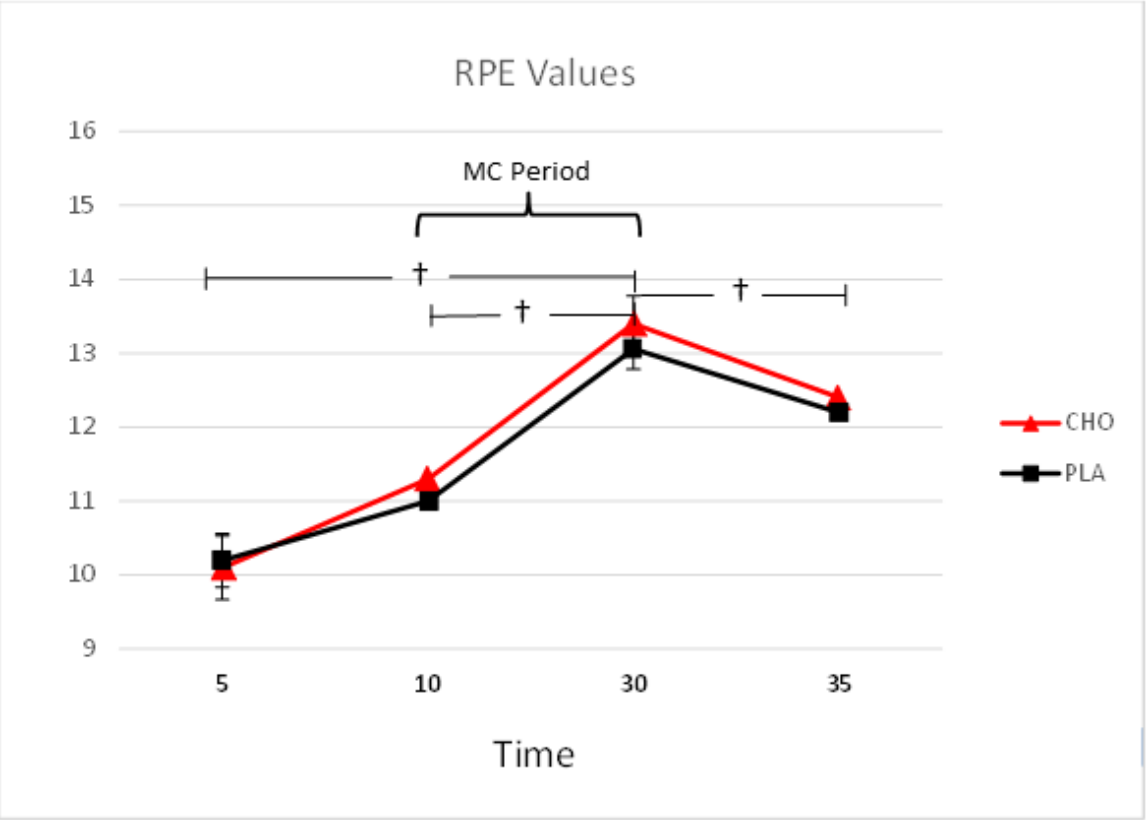

Figure 4.3 Changes in rating of perceived exertion (RPE) values over time.

PLA = placebo condition; CHO = carbohydrate condition. MC = mental challenge. The X axis (time) shows time in min after start of exercise.

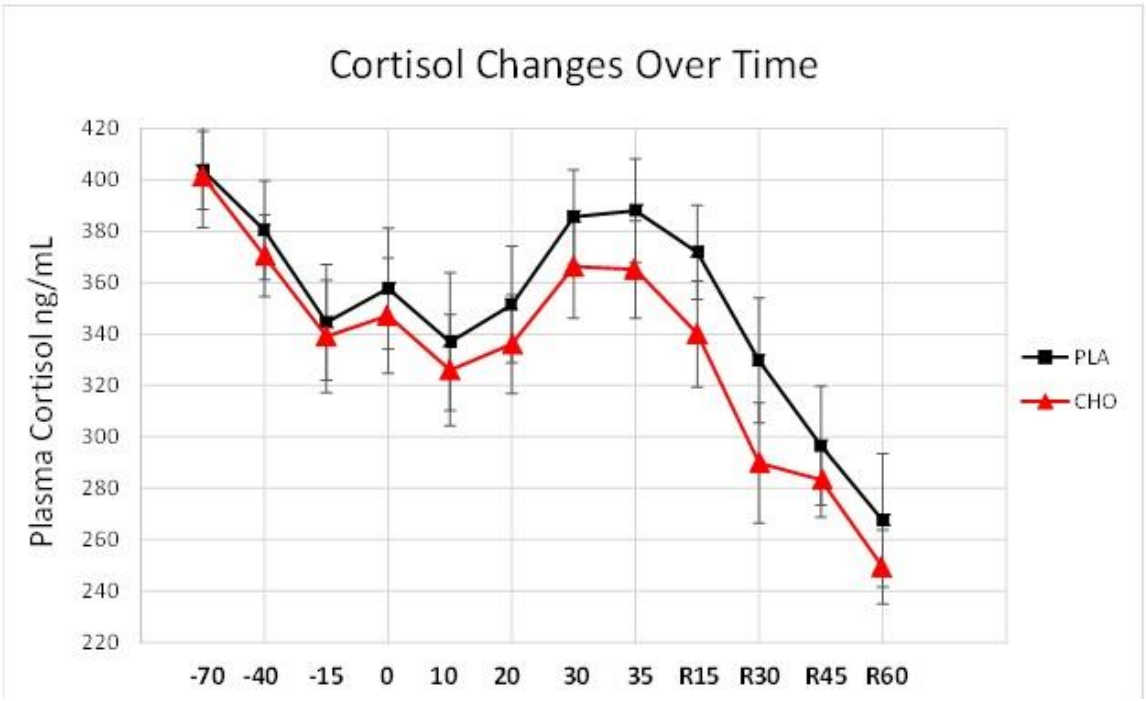

Figure 4.4 Changes in plasma cortisol concentrations over time.

PLA = placebo treatment; CHO = carbohydrate treatment. -70 = 70 min before exercise. 0 = start of exercise. 35 = end of exercise. R = post exercise recovery period.

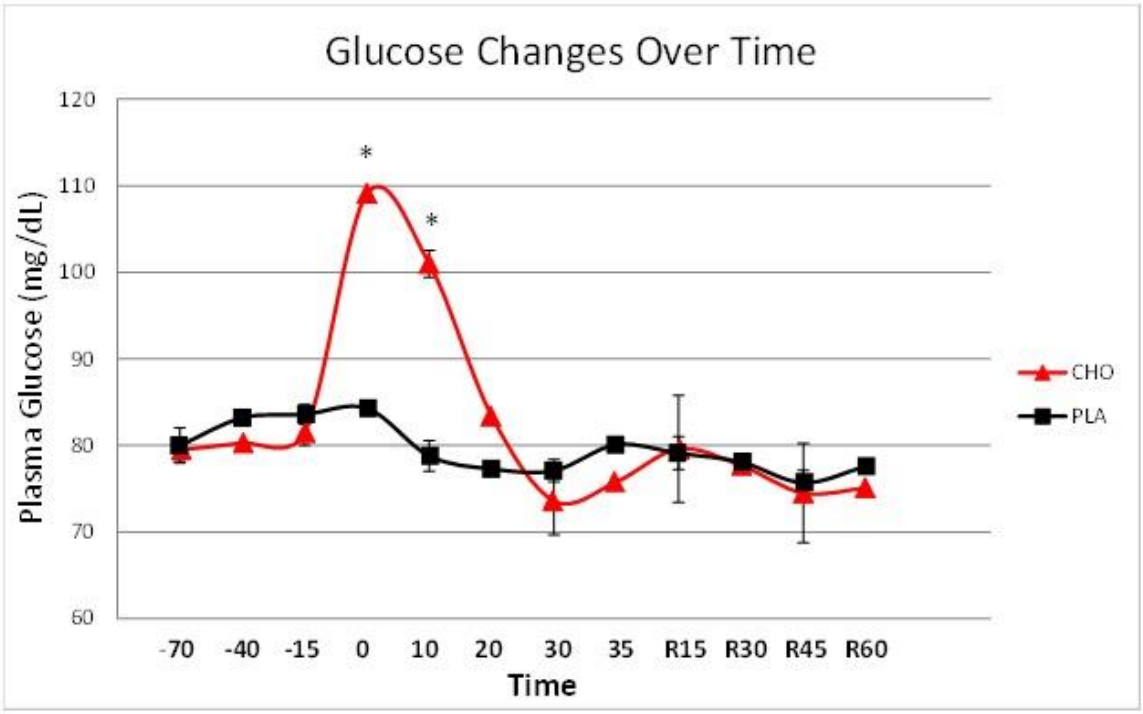

Figure 4.5 Plasma glucose responses before, during, and after the dual-concurrent stress (DCS).

*Indicates a significant difference between treatments ($p < 0.05$). PLA = placebo condition; CHO = carbohydrate condition. -70 = 70 min before exercise. 0 = start of exercise. 35 = end of exercise. R = post exercise recovery period

CHAPTER V

CONCLUSIONS

The main findings of this investigation were that (1) CORT production tended to be lower during and after a DCS challenge when CHO was ingested prior, and (2) the DCS challenge was effective at increasing anxiety and acting as an effective *stressor*. In order to achieve the former, it is imperative that the latter is achieved which ensures that the mental and physical stressors are increasing anxiety of participants as well as increasing stress of the HPA axis. This was an important aspect of this project in order to have external validity regarding the efficacy of CHO ingestion prior to DCS scenarios. However, additional trials are needed to further investigate this acute nutritional intervention, especially since there was a large amount of variance between subjects in terms of CORT production.

It was expected to observe elevated CORT production immediately upon participant's arrival to the lab which coincides with previous reports that CORT levels tend to be elevated first thing in the morning. However, CORT levels could have also been elevated due to increased anxiety from participants associated with the IV insertion procedure. The purpose of the seventy min sit prior to exercise was to make an attempt to (1) decrease variance between subjects in CORT levels immediately prior to the DCS and (2) reduce the elevated CORT levels associated with the aforementioned. There was a consistent trend for decreasing CORT levels during the seventy min sit which was

expected. Further, the CORT levels 60 min after exercise were lower than before the start of exercise which indicates that the participants still had a slight amount of pre-exercise anxiety before initiating exercise. This is a possible limitation to the study since we were not able to establish a true baseline for CORT levels even when incorporating a 70 min sit prior to exercise. However, it is likely unachievable to achieve such in a laboratory setting since participants are expected to have a small amount of pre-testing anxiety.

One of the most interesting aspects of this investigation is that both perceived exertion and anxiety levels of the participants were highest immediately upon completion of the DCS challenge. Since RPE and SAI levels decreased after the end of the mental stress challenge even with no change in physical workload, it is expected that exercise alone at 60% VO_2 peak would not have elicited such large increases in CORT levels; however, this study did not include an exercise alone condition to make such a comparison. The purpose of this study was to investigate the effect of CHO ingestion on CORT production to a DCS challenge therefore we are not able to determine if similar findings would be reported during exercise or mental stress alone. There was a trend for lower SAI levels during the DCS when CHO was ingested prior however, the reduction was not significant.

In terms of future research, since firefighters carry additional external loads (personal protective equipment), future studies should investigate the effects of physical load as well as a third added stressor (heat) on cardiovascular responses to combined physical and mental stress. Potential exercise or dietary interventions could serve as a protective mechanism in such high stress scenarios; however, there is a lack of scientific support behind this claim. This study did not include an analysis on pro-oxidant

accumulation from the DCS challenge which should also be included in future studies. Finally, the findings from this investigation are not limited to individuals that work in high stress occupations but to all individuals that are chronically exposed to concurrent mental and physical stress.

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APPENDIX A

IRB APPROVAL

November 26, 2014

Matthew McAllister
Kinesiology
Mailstop 9575

RE: Your application dated regarding study number 14-343: Effect of Carbohydrate Supplement on Cardiovascular and Metabolic Responses to Dual Concurrent Stressors

Dear Mr. McAllister:

Thank you for your response to requests from a prior review of your application for the new study listed above. Your response was reviewed by the Human Research Protections Program.
This email serves as official documentation that your application is now fully approved.
You are granted permission to conduct your study as most recently described effective immediately. The study is subject to continuing review on or before 10/15/2015, unless closed before that date.
Please note that any changes to the study as approved must be promptly reported and approved. Some changes may be approved by expedited review; others require full board review. Contact Nicole Morse (nmorse@orc.msstate.edu or call 662-325-5220) if you have any questions or require further information.

Sincerely,
Nicole Morse, CIP
IRB Compliance Administrator

APPENDIX B

PARTICIPATION AND HEALTH HISTORY QUESTIONNAIRE

PARTICIPATION AND HEALTH HISTORY QUESTIONNAIRE

Complete each question accurately. All information provided is strictly confidential.

Part I: Participant Information

Name (Print) _____ Home Phone # _____

Current Mailing Address _____ Work/Cell Phone # _____

Personal Physician _____ Email Address _____

Emergency Contact (relationship) _____ Emergency Contact Phone # _____

Gender: _____ Female _____ Male Date of Birth: _____

Height _____ Weight _____ Age _____

Part II. Health History

List any physical injuries or limitations that you have at this time: _____

_____

Have you ever been diagnosed as having any cardiovascular abnormalities?

_____ Yes _____ No

If yes, what was diagnosed and when was the diagnosis conducted? _____

_____

Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

Heart Attack	Bypass surgery	Sickle-Cell Anemia
Heart Palpitations	Arrhythmia	Chest pain
Shortness of breath	Stroke	Anemia
Heart valve problems	Heart murmur	Heart rhythm abnormalities

Do you have any form of respiratory (breathing) ailments? Please circle those that apply.

Asthma	COPD	Common cold
Emphysema	Bronchitis	

Have you been diagnosed with any of the following? If yes, please circle the appropriate ailment.

Rheumatic fever	High blood pressure	Kidney/Liver disease
Obesity	Diabetes	High Cholesterol
Color Blindness	Hemophilia	

Does anyone in your family have any of the conditions listed above? If yes, please list relation to family member and problem: _____

_____

Is your mother living? _____ Yes _____ No (age at death _____; cause_____)

Is your father living? _____ Yes _____ No (age at death _____; cause_____)

Do you have any allergies (latex, food, drug, etc.)? _____ Yes _____ No

If yes, please list: _____

_____

Have you had a prior graded exercise test? _____ Yes _____ No

If yes, when and what were the results? _____

_____

Have you ever experienced any adverse responses during or after exercise (i.e. dizziness, difficulty breathing, racing heart beat, fainting)? _____ Yes _____ No

If yes, what were the symptoms? _____

_____

Have you ever been diagnosed with a psychological disorder? _____ Yes _____ No

Part III. Health Related Behavior

Do you smoke? _____ Yes _____ No

If yes, indicate number of cigarettes per day?

_____ Less than ½ a pack _____ 1 pack ____ Greater than 1 pack

Do you drink alcohol? _____ Yes _____ No

If yes, indicate number of alcoholic beverages per week?

_____ Less than 10 _____ 10 ____ Greater than 10

Do you exercise regularly (30 min, 3 times per week)?

_____ Yes _____ No

If so, what exercises do you participate in regularly? _____

_____

Have you been diagnosed with hearing problems? _____ Yes ____ No

Have you been diagnosed with vision problems? ____ Yes _____ No

Have you recently (within 1 month) experienced a major life event (i.e., death in family, divorce; wedding; birth of a child)? _____ Yes _____ No

Have you donated blood or plasma within the previous month?

_____ Yes _____ No

How would you rate your ability to add and subtract numbers?

_____Very capable _____Capable _____Not very capable

How would you rate your comfort level with adding and subtracting numbers?

___ Very much at ease ____ At ease ___ Somewhat at ease ___ Very Uncomfortable

Are you taking any medications (prescription/nonprescription) or supplements?

_____ Yes _____ No

If yes, please list: _____

_____

Are you allergic to artificial sweeteners? ____ Yes _____ No

If yes, please list which ones: _____

_____

APPENDIX C

INFORMED CONSENT

Consent to Participate in a Research Study

Effect of Carbohydrate Supplement on Cardiovascular and Metabolic Responses to Dual Concurrent Stressors

Investigators: Matthew J. McAllister

Mississippi State University
Department of Kinesiology 124
McCarthy Gymnasium
Mississippi State, Mississippi 39762
(901)484-0471

Description: You are being asked to voluntarily participate in research designed to investigate the effects of carbohydrate ingestion on a combination of physical and mental stress. Your participation will include coming to the Applied Physiology Lab (Room 131, McCarthy Gymnasium) for four testing sessions. The purpose of this project is to investigate the potential ability of exogenous CHO ingestion to attenuate cortisol production to dual concurrent stressors. The specific aim of this study is to investigate the impact of the ingestion of a carbohydrate beverage on markers of cardiovascular health in response to a dual stress challenge. Below is an explanation of what you will be asked to do during those three sessions.

During the first session you will be introduced to the research team, complete a medical history questionnaire, a short questionnaire regarding your feelings and thoughts during the previous month, and we will discuss the project with you in further detail. During the second session you will perform a VO2 peak test as well as participate in a short practice run of the stress protocol (mental challenges performed during exercise). The mental challenges consist of a color-word task as well as mental arithmetic. For the color word challenge you will be shown the name of a color that may conflict with the font color of the word displayed. You will subsequently be asked to recall the color of the font. For the arithmetic you will be asked to complete a series of mathematical subtractions. In addition, throughout this testing session you will be asked to complete a short questionnaire addressing how nervous you are and two other questionnaires addressing how hard you are working at the task. For the final component of this session you will be asked to participate in an assessment of your aerobic (cardiovascular) fitness. You will be asked to ride a stationary bike that will periodically increase in workload (resistance) until you reach exhaustion. The initial workload will start at 100W. Workload will be increased by 50W every 3 minutes until minute 14. At minute 14, the workload will increase by 50W every minute. During this ride we will be collecting your expired air. You will ride until exhaustion. During this ride you will breathe into a mouthpiece while wearing a nose clip. To monitor heart rate the researchers will use a heart-rate monitor, which will require you to wear an adjustable strap around your chest. The introductory information should take approximately 15 min, the first testing component should take approximately 10 minutes, and the final fitness assessment should take no more than 20

minutes. Thus, the total time for this first session should be approximately 15 minutes. The total time for the second session should be 45-60 minutes.

If you qualify for the study, in the remaining two sessions you will be asked to complete a 35- minute cycle rides at a moderate intensity (60% of your aerobic capacity [fitness] level). Again, you will be asked to breathe into a mouthpiece while wearing a nose clip. You will ingest a carbohydrate beverage or sweetened water 15 minutes prior to this exercise test. The sweetened water will contain no calories and will contain the following ingredients: water, citric acid, gum arabic, potassium citrate, salt, sodium citrate, potassium phosphate, sodium phosphate, sucralose and natural and artificial flavor. During these sessions you will participate in the same tasks as those described for the first session for 20 minutes during each ride. For both of these sessions you will exercise for 35 total minutes at 60% of your aerobic capacity while performing mental challenges on a computer. The only difference between sessions will be the type of beverage you ingest. It will be either a carbohydrate beverage or sweetened water. You will report to the lab at 7:00 AM after fasting for at least eight (8) hours, and the total time required for these sessions will be approximately 3.5 hours.

During the third and fourth testing sessions, a hypodermic needle will be used to insert a venous catheter (small tube) into a vein in your arm by a trained professional, and blood will be drawn periodically throughout the sessions. The venous catheter will allow us to collect samples of your blood without further needle "sticks".

We will collect approximately 150ml (~3/4 cup) of blood (in 12ml [~1 tablespoon] samples) during each research session. This is less than half of the amount of blood that is collected during a blood donation. The blood will be used to measure hormonal responses to the exercises. During the research session, we will replace the blood removed with an equal amount of physiologic (normal) saline in order to prevent dehydration and maintain a normal blood volume.

Additionally, we will wait at least a week between your second and third sessions.

At the conclusion of the third and fourth sessions, we will provide you with fruit juices and breakfast foods (boxed cereal, granola/breakfast bars, etc.). We will also have bottled water and fruit juice available for you at all times during the testing sessions should you feel lightheaded or dizzy due to low blood sugar or blood pressure levels that may occur due to the blood collection or exercise. We will also provide you with feedback on your results at the end of these sessions. Your feedback will be based solely on your performance on these testing sessions.

You should know that we are looking for specific characteristics in our subjects. These include;

a) having no hearing or vision problems, b) having no history of psychological disorder or chronic illness, c) the use of English as a first language, d) not taking any medications, supplements, or tobacco products, e) consuming an average of less than 10 alcoholic beverages per week, f) not having experienced any recent major life events (e.g. death in family, divorce, wedding) in the past 6 months, g) not being math phobic, h) not having any class exams or other evaluated class activities (presentations, assignments due) on the days of testing, and i) not having donated blood within the previous month. This information will be gathered during the initial testing session.

Risks: Your participation in this study may involve some risks. The most common are the possibility of feeling a "shortness of breath", dizzy/lightheaded, and general fatigue as well as possible fainting during one or all of the testing procedures. If you cannot tolerate the feeling of "shortness of breath", dizziness, or fatigue, you can ask the researcher to stop at any point during the research. In addition, it is common for the mental challenges associated with this research to cause you to experience (for short periods of time) feelings of frustration and anxiety. During the research sessions, you may experience discomfort during the insertion of the venous catheter that will be utilized for blood sample collection. This procedure may require more than one needle stick for optimal catheter placement. The possibility of infection exists, but will be minimal due to the use of sterile techniques. A phlebotomy trained individual will administer these procedures to minimize discomfort and provide optimal care. Instructions for care of the insertion site will be given to you at the conclusion of each session.

Benefits: The maximal oxygen consumption test will provide you with an accurate assessment of your aerobic (cardiovascular) fitness. This is a test that often requires $100 in research cost to have conducted; however, you will receive this test for free. You will also receive information on how your body responds to cognitive challenges. You will also be provided with information on exercise prescription for increasing your cardiovascular fitness levels if you so desire.

Confidentiality: The results of the tests and all the associated records will be kept strictly confidential, and only members of the investigative team will have access to these documents. If your individual test results are reported at a scientific meeting or published in a scientific journal, only your assigned participant number, rather than full name, will be used.

On occasion, we may take photos or videotape you during your participation in the study for presentations at conferences or in manuscripts. We will make every attempt to keep you from being recognized in the video and/or photos. We will inform you if we wish to photograph or record you. You may choose to participate in this research, while opting out of being photographed or recorded by initialing your preference below.

*Please note that these records will be held by a State entity and therefore are subject to disclosure if required by law.

I agree to allow photographic and videographic recording of my participation in the study entitled, "*Effect of Carbohydrate Supplement on Cardiovascular and Metabolic Responses to Dual Concurrent Stressors"*.

CHAPTER VI_____YES NO

Research Questions: If you should have any questions about this research project, please feel free to contact Matthew McAllister (901)-484-0471. This study has been reviewed by the Mississippi State University Institutional Review Board (IRB). The IRB has determined that this study meets the ethical obligations required by federal law and University Policies. If you have any questions, concerns or reports regarding this study, please contact the MSU Office of Research Compliance at 662-325-3294.

Approved: 12/19/14 Expires: 10/15/15

IRB # 14-343

Compensation for Illness or Injury: You understand that you are not waiving any legal rights or releasing the institution or their agents from liability from negligence. In addition to reporting an injury to Matthew McAllister (901-484-0471) and to the Office of Research Compliance (662-325-3294), you may be able to obtain limited compensation from the State of Mississippi if the injury was caused by the negligent act of a state employee where the damage is a result of an act for which payment may be made under §11-46-1, et seq. Mississippi Code Annotated 1972. To obtain a claim form, contact the University Police Department at *MSU UNIVERSITY POLICE DEPARTMENT, Williams Building, Mississippi State, MS 39762, (662) 325-2121.*

Right to Withdraw: You do not have to take part in this study. If you start the study and decide that you want to withdraw, you need only to inform Matthew McAllister or anyone else on the research team. You can do this in person, by letter, or by telephone at the Department of Kinesiology, Mississippi State University, MS 39762, or 662-325-2963. Whether or not you choose to participate will not affect your standing within the Department Kinesiology, or with the University, and will not cause you to lose any benefits to which you are entitled. For additional information regarding your rights as a research subject, please feel free to contact the MSU Office of Research Compliance at 662-325-3294.

Statement of Consent: I have read the above information. I have been given a copy of this form. I have had an opportunity to ask questions, and I have received answers. I consent to participate in the study.

You will be given a copy of this form for your records

Signature:_____Date: _____

Signature of Investigator:_____Date: _____

MSU HRPP Full Accreditation	Approved: 12/19/14 Expires: 10/15/15 IRB # 14-343