THE COMPARISON OF HIGH-INTENSITY INTERVAL EXERCISE VS. CONTINUOUS MODERATE EXERCISE ON C1Q/TNF-RELATED PROTEIN-9 EXPRESSION AND FLOW-MEDIATED VASODILATION IN OBESE INDIVIDUALS

by

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Chun-Jung Huang, Department of Exercise Science and Health Promotion, and has been approved by the members of his supervisory committee. It was submitted to the faculty of the College of Education and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

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Title: The Comparison of High-Intensity Interval Exercise vs. Continuous Moderate Exercise on C1q/TNF-Related Protein-9 Expression and Flow-Mediated Vasodilation

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The primary purpose of this study was to investigate the effect of acute high-intensity interval exercise (HIIE) vs. continuous moderate-intensity exercise (CME) on serum CTRP9 and brachial FMD responses in obese and normal-weight subjects. Sixteen participants (9 obese and 7 normal-weight) completed HIIE and CME in a randomized fashion. Our results showed a significant time effect for CTRP9 immediately following acute HIIE and CME in both groups. Furthermore, both significant treatment by time and group by time interactions for FMD were observed following both exercise protocols, with greater CME-induced FMD response in obese subjects than normal-weight subjects. Additionally, a positive correlation in percent change (baseline to peak) between CTRP9 and FMD was observed following acute CME. These findings support acute CME for improvement of endothelial function in obesity. Furthermore, the novel results from this study provide a foundation for additional examination of the mechanisms of exercise-mediated CTRP9 on endothelial function.

THE COMPARISON OF HIGH-INTENSITY INTERVAL EXERCISE VS. CONTINOUS MODERATE EXERCISE ON C1Q/TNF-RELATED PROTEIN-9 EXPRESSION AND FLOW-MEDIATED VASODILATION IN OBESE INDIVIDUALS

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CHAPTER I: INTRODUCTION

Obesity is a national and global epidemic as its prevalence has consistently increased for the past three decades (Wise, 2014). The obesity-associated metabolic profile has been linked to an increased risk of numerous inflammatory diseases, including diabetes and cardiovascular disease (Sattar et al., 2003). The processes of these cardiovascular consequences include infiltration of inflammatory leukocytes (e.g., macrophages) to the vessel wall and apoptosis of smooth muscle cells, leading to endothelial dysfunction (Rocha & Libby, 2009). The resulting impairment of endothelium-dependent vasodilation or vasomotor function is one of the earliest subclinical stages in the atherosclerotic process (Singhal, 2005).

A common method of assessing endothelial and vasomotor function is the measure of flow mediated dilation (FMD), which is facilitated by the release of endothelial relaxing factors (e.g., nitric oxide [NO]), resulting in vasodilation (Henrion, 2005). Furthermore, obesity-related inflammation has a negative impact on the endothelium via down-regulation of vasodilatory signaling pathways (Meyer, Kundt, Steiner, Schuff-Werner, & Kienast, 2006), such as the AMP-activated protein kinase (AMPK) mechanism (Zheng et al., 2011). Specifically, AMPK promotes endothelial cell NO synthase (eNOS) phosphorylation through direct or indirect protein kinase B (Akt) activation (Uemura et al., 2013). A recent novel adipocytokine, C1q-TNF-related protein-9 (CTRP9), has been shown to increase

eNOS activation via the AMPK-Akt-eNOS mechanism in human umbilical vein endothelial cells (Zheng et al., 2011). Importantly, CTRP9 has been shown to be down-regulated in obese mice (Uemura et al., 2013) and patients with insulin resistance (Hwang, Oh, Park, & Park, 2014; Wang et al., 2015). Serum CTRP9 is also inversely correlated with visceral fat in humans (Hwang et al., 2014). Interestingly, CTRP9 levels are elevated in obesity and significantly decrease following weight loss surgery, suggesting that CTRP9 may play a compensatory role in obesity, similar to that of insulin (Wolf et al., 2016). Taken together, these findings suggest the important role of CTRP9 on the modulation of endothelial function, especially in obese individuals.

Regular physical activity has been shown to improve inflammation (Adamopoulos et al., 2001; Ringseis, Eder, Mooren, & Krüger, 2015), as well as vascular and endothelial function in cardiac patients (Rognmo, Hetland, Helgerud, Hoff, & Slørdahl, 2004). A greater improvement in aerobic capacity (peak oxygen consumption) was observed in patients with coronary artery disease following ten weeks of high-intensity interval exercise (HIIE) training than continuous moderate-intensity exercise (CME) (Rognmo et al., 2004). Furthermore, Guimarães and colleagues (2010) demonstrated that HIIE training lowered resting blood pressure in hypertensive patients, suggesting a greater improvement in endothelial and/or vasomotor function . A recent review and meta-analysis by Ramos, Dalleck, Tjonna, Beetham, & Coombes (2015) concluded HIIE training improves FMD more than CME training. Importantly, HIIE training has been shown to promote a greater enhancement in insulin sensitivity in both fat and skeletal muscle (Tjønna et al., 2008) and endothelial function (brachial artery FMD) compared to CME (Wisløff et al., 2007). Additionally, FMD was shown to be

elevated up to two hours following acute HIIE, whereas CME remained unchanged in healthy adolescents (Bond, Hind, Williams, & Barker, 2015). Finally, acute HIIE showed a similar improvement in FMD in patients with coronary artery disease after one hour of recovery compared to acute CME while a higher total work was observed in the CME (Currie, McKelvie, & MacDonald, 2012). These findings indicate acute HIIE could be a more time effective method to provide comparable cardiovascular health benefits (e.g., increased FMD and CTRP9). However, the exact mechanisms regarding exercise-mediated FMD and CTRP9 (either HIIE or CME) in obesity still remains to be studied.

Therefore, the primary purpose of this study was to investigate the effect of acute HIIE vs. CME on serum CTRP9 and brachial artery FMD responses in obese and normal-weight subjects. Furthermore, this study also examined the association of exercise-mediated CTRP9 with FMD following acute HIIE and CME in both groups. It was hypothesized that obese subjects would elicit greater serum CTRP9 and FMD responses than normal-weight subjects following both exercise protocols. These responses were projected to be equivalent between acute HIIE and CME in both groups. Finally, increased levels of serum CTRP9 were thought to be positively correlated with FMD in both exercise groups.

CHAPTER II: REVIEW OF LITERATURE

Introduction

Obesity has become one of the leading causes of morbidity and mortality in the United States with the prevalence of approximately 35% for men, 40.4% for women, and 17% for youths (N. B. Johnson, Hayes, Brown, Hoo, & Ethier, 2014; Zylke & Bauchner, 2016). Obesity in individuals 19 to 64 years of age attributes to 2.8% of all medical costs (Long, Reed, & Lehman, 2006). Additionally, the estimated medical costs of obesity from 2008 were $147 billion (Finkelstein, Trogdon, Cohen, & Dietz, 2009). Obesity contributes to an array of inflammatory related conditions, including cardiovascular disease (Withrow & Alter, 2011). The processes of these cardiovascular consequences include the infiltration of inflammatory leukocytes to the vessel wall and apoptosis of smooth muscle cells, leading to decreased vascular function (Rocha & Libby, 2009). A recent novel adipocytokine, C1q-TNF-related protein-9 (CTRP9), has been shown to increase eNOS activation via the AMPK-Akt-eNos mechanism in human umbilical vein endothelial cells (Zheng et al., 2011), suggesting CTRP9 plays an important role in vascular function. Exercise has been shown to decrease inflammation in obese individuals and improve endothelial function (Joris, Zeegers, & Mensink, 2015; Ringseis et al., 2015). More recently, high-intensity interval exercise (HIIE) has become popular for exercise interventions in the obese population (Wallman, Plant, Rakimov, & Maiorana, 2009). Specifically, HIIE has been shown to be more time-efficient when compared to moderate-intensity continuous exercise while providing similar health

benefits (Gibala & McGee, 2008). Therefore, this chapter will mainly discuss the impact of obesity on inflammation and endothelial function as well as HIIE benefits for cardiovascular health when compared to CME.

Obesity and Inflammation

Obese individuals are typically in a state of low-grade chronic inflammation (Hotamisligil, 2006). This obesity-related inflammation is often termed metaflammation (metabolically triggered inflammation) (Hotamisligil, 2006). Obesity has been linked to many inflammatory conditions such as type 2 diabetes, stroke, coronary artery disease, gallbladder disease, hyperlipidemia, hypertension, and some types of cancer- all of which place a substantial economic burden on the health care system (Withrow & Alter, 2011). Obese individuals are typically consuming an excess amount of calories, causing immune dysfunction with higher observations of infections and impaired wound healing (Marti, Marcos, & Martinez, 2001). Marti et al. (2001) also conveys that with excess body fat there are increases in some leucocyte counts, such as increased neutrophils and monocytes, but lower T-cell and B-cell mitogen-induced proliferation. Additionally, it has been shown that following a vaccination, the production of antibodies is blunted in obese individuals (Sheridan et al., 2012).

This obesity mediated inflammation can be explained by increased adipose tissue storage which causes adipocyte apoptosis triggered macrophage infiltration, as well as neutrophil, $CD4^+$ and $CD8^+$ T cell recruitment consequently leading to insulin resistance within the adipose tissue (Bouloumie, Casteilla, & Lafontan, 2008; Cinti et al., 2005). Following macrophage infiltration, reactive oxygen species (ROS) can be released, signaling the production of pro-inflammatory cytokines. The overproduction of ROS has

been linked to oxidative stress and inflammation (Bulua et al., 2011). Additionally, pro-inflammatory cytokines associated with obesity such as TNF-a, IL-6, and C-reactive protein (CRP) cause peripheral blood mononuclear cells (PBMCs) to be in a pro-inflammatory state (Ghanim et al., 2004). Importantly, this pro-inflammatory profile also causes a switch from the M2 (anti-inflammatory) to the M1 (pro-inflammatory) phenotype of macrophages (Lumeng, Bodzin, & Saltiel, 2007). Similarly, the ratio of $CD8^+$ to $CD4^+$ T cells has been shown to increase with obesity, this is important because $CD4^+$ regulatory T-cells secrete anti-inflammatory cytokines that inhibit macrophage migration (Feuerer et al., 2009; Nishimura et al., 2009).

Adipocytes secrete adipokines that have immune-modulatory actions (Trayhurn & Wood, 2004). Important adipokines include leptin and adiponectin. Leptin is known to stimulate monocyte proliferation and differentiation into macrophages. In addition, it modulates the activation of natural killer cells (NK cells) or induces the release of pro-inflammatory cytokines (TNF-a, IL-6, and IL-12) (Marti et al., 2001; Tilg & Moschen, 2006). Leptin modulates the central nervous system (CNS) to stimulate satiety and energy expenditure. Importantly, circulating levels of leptin increase with body fat. Therefore, obesity is associated with leptin resistance resulting in the activation of immune cells mentioned above (de Heredia, Gómez-Martínez, & Marcos, 2012). Adiponectin, an anti-inflammatory and insulin-sensitizing hormone, is inversely related to body weight and has opposite immune-modulatory actions from that of leptin. In macrophages it inhibits phagocytosis and the production of TNF-a (de Heredia et al., 2012). Adiponectin also inhibits the differentiation of monocytes and the production of endothelial adhesion molecules (Koerner, Kratzsch, & Kiess, 2005). Additionally,

adiponectin induces the production of IL-10 and IL-1 receptor antagonist (IL-1RA) (Tilg & Moschen, 2006).

Exercise is a means of combating obesity by counteracting the positive energy balance (Park, Myers, & Vieira-Potter, 2014). Exercise can also mediate the metainflammation associated with obesity (Ringseis et al., 2015). This can been seen with regular exercise to modify metabolic hormones which may counteract the chronic inflammation and obesity-related conditions (McMurray & Hackney, 2005). Increased physical activity has been shown to have an anti-inflammatory effect (Jennersjö et al., 2012). A study by Arikawa, Thomas, Schmitz, & Kurzer (2011) demonstrated that 16 weeks of aerobic exercise significantly decreased CRP in young women, even more so for those who were obese. A similar study showed that aerobic exercise over a period of 10 months decreased CRP, IL-6, and IL-18 significantly (Kohut et al., 2006). Other exercise modes have been shown to decrease inflammatory biomarkers including resistance exercise. Olson, Dengel, Leon, & Schmitz (2007) demonstrated that with 1 year of resistance exercise CRP levels deceased significantly and adiponectin levels improved. Interestingly, another study with just 10 weeks of resistance training also showed a significant decrease in CRP (Donges, Duffield, & Drinkwater, 2010). A study with older adults demonstrated that greater physical activity was associated with lower systemic concentrations of IL-6 (Nicklas et al., 2008).

Obesity and Vascular/Endothelial Dysfunction

The inflammatory profile linked with obesity can cause cardiovascular disease; this is caused by the infiltration of macrophages into the vessel wall and cell death of vascular smooth muscle cells leads to impaired vascular function (Rocha & Libby, 2009).

Endothelial cells have an important role in maintaining cardiovascular homeostasis by providing a physical barrier between the vessel wall and lumen, as well as secreting a number of mediators. These mediators regulate platelet aggregation, coagulation, fibrinolysis and vessel tone (Avogaro & de Kreutzenberg, 2005). Additionally, endothelial cells mediate vasoconstriction by releasing endothelin-1 and thromboxane A2 and vasodilation by secreting nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF) (Vallance, 2001). Nitic oxide is the major modulator of endothelium-dependent vasodilation in conduit arteries and is classically known as the endothelium derived relaxing factor (EDRF) (Vallance, 2001). Nitric oxide is synthesized by endothelial nitic oxide synthase (eNOS) which relaxes the smooth muscle cells surrounding the vessel wall (Vallance, 2001).

The endothelium stores L-Arginine which is the precursor of NO. The endothelial cells can convert L-Arginine to L-Citrulline and NO. Specifically, within the endothelial cells, nitric oxide synthase (NOS) can access stored L-arginine (Simon et al., 2003). L-Arginine is converted to NO by a family of NOS isoforms, such as eNOS, neuronal (nNOS), and inducible or inflammatory (iNOS). The eNOS activity can be regulated in a number of ways including post-translational modifications. Interestingly, these modifications occur through the phosphorylation of Ser1179, increasing the activity of the enzyme (Fulton et al., 1999). The phosphorylation of this site can be done by several kinases such as protein kinase A, protein kinase C, and serine/threonine kinase Akt (Gratton, Bernatchez, & Sessa, 2004). The increased activity of eNOS can convert L-Arginine to NO and L-Citrulline. Nitric oxide can then mediate the vascular smooth muscle cells to relax causing vasodilation (maintaining basal tone) (Avogaro & de

Kreutzenberg, 2005). Importantly, NO also inhibits platelet adhesion, activation, secretion and aggregation and promotes platelet disaggregation (T. J. Anderson, 2004). Endothelial-derived NO also inhibits leukocyte adhesion to the endothelium which inhibits smooth muscle cell migration and proliferation (Avogaro & de Kreutzenberg, 2005). Unfortunately, inflammation caused by obesity such as increased TNF-a have been shown to inhibit transcription, and post-transcriptional eNOS gene expression (H. D. Anderson, Rahmutula, & Gardner, 2004).

An additional consequence of the chronic low-grade inflammation associated with obesity is the damage it does to endothelial cells (Avogaro & de Kreutzenberg, 2005). Elevated low-density lipoproteins (LDL) can diffuse into the intima (Hansson, 2005). During lesion formation, endothelial cells present cell adhesion molecules (CAMs) such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) which recruit circulating leukocytes such as monocytes during lesion formation. However, healthy endothelial cells down-regulate these adhesion molecules (Libby, Ridker, & Maseri, 2002). Compromised endothelial cells release monocyte chemotactic protein 1 (MCP-1) as well as macrophage colony stimulating factor (M-CSF) which attract circulating mononuclear cells and cause their differentiation into macrophages in the vessel wall (Libby et al., 2002). After which, these macrophages release pro-inflammatory cytokines (IL-1, IL-6, TNF-a), which exacerbates inflammation by causing other local cells to release additional inflammatory cytokines, subsequently leading to endothelial dysfunction (Ribeiro, Alves, Duarte, & Oliveira, 2010).

The impairment of the endothelium-dependent vasodilation is one of the earliest sub-clinical stages in the atherosclerotic process (Singhal, 2005). A common method of

assessing endothelial function is the measure of flow-mediated dilation (FMD), which mediates the release of endothelial relaxing factors (NO), resulting in vasodilation (Henrion, 2005). Flow-mediated dilation has been validated as an endothelium-dependent and NO-specific index of endothelial function (Kooijman et al., 2008; Thijssen et al., 2010). The process of flow-mediated dilation is initiated by shear-stress to an imaged artery (using ultrasound) by inducing reactive hyperemia (using occlusion) to the resultant diameter change (Thijssen et al., 2010). Shear-stress activates a signaling cascade that results in vasodilator production (NO). The vasodilators produced are subject to the nature of the shear stress stimulus and the type of endothelium stressed (K. Pyke et al., 2010; K. E. Pyke & Tschakovsky, 2005). Shear stress can modulate the endothelial cells to produce eNOS and then nitric oxide, which then diffuses into smooth muscle cells (Thijssen et al., 2010). However, NO may react with ROS resulting in decreased bioavailability (Rush, Denniss, & Graham, 2005). In vascular smooth muscle cells vasodilators (NO) trigger a signaling cascade to lower calcium concentration to induce vasodilation. Vessel wall factors may influence the vessel wall change (Lind, 2007). FMD is quantified as the percent change in vessel diameter from baseline prior to occlusion and the resultant reactive hyperemia (shear-stress stimulus) stimulated peak diameter (Thijssen et al., 2010).

A low measurement of flow-mediated dilation has been identified as an independent risk factor for future cardiovascular events (Rossi, Nuzzo, Origliani, & Modena, 2008). Furthermore, FMD have been reported to have strong associations with cardiovascular disease (Celermajer et al., 1992; Enderle et al., 1998; Takase et al., 1998). Importantly, obesity has been shown to impair FMD (Davison et al., 2011), while other

studies show that increased body fat has been associated with decreased FMD (Keogh, Brinkworth, & Clifton, 2007; Sciacqua et al., 2003; C. Y. Wong et al., 2006; Wycherley, Brinkworth, Noakes, Buckley, & Clifton, 2008). The chronic inflammation associated with obesity has a negative impact on the endothelium to down-regulate vasodilatory signaling pathways (Meyer et al., 2006). These include the AMP-activated protein kinase (AMPK) mechanism, contributing to NO production (Zheng et al., 2011). Specifically, AMPK mediates endothelial cell NO synthase (eNOS) phosphorylation through direct or indirect protein kinase B (Akt) activation (Uemura et al., 2013).

Exercise –mediated Endothelial Function in Obesity

A. Aerobic and Resistance Exercise

Along with obesity, reduced cardiovascular fitness have been shown to be associated with lower endothelial function (FMD) (Lippincott et al., 2008). Exercise can be used to improve endothelial function by lowering weight and potentially improving cardiovascular fitness (Joris et al., 2015). It was estimated that with each 10 kg drop in weight there is an associated 1.11% improvement in fasting FMD (Joris et al., 2015). A study by Clarkson et al. (1999) demonstrated that with 10-weeks of aerobic exercise combined with anaerobic exercise (upper-body strength exercises) can improve FMD in healthy males. Clarkson et al. (1999) postulated that increased NOS expression was responsible for the improvement in endothelial function accessed by FMD. Acute high-intensity and moderate-intensity exercise have been shown to increase FMD in a time-dependent manner, initially (less than 30 minutes) following exercise FMD decreases (Dawson, Green, Cable, & Thijssen, 2013; B. D. Johnson, Padilla, & Wallace, 2012). Following one hour post exercise FMD begins to normalize (Birk et al., 2013). Many

studies have demonstrated an improvement in FMD one-hour post exercise (Cosio‐Lima et al., 2006; Goel et al., 2007; Harris, Padilla, Hanlon, Rink, & Wallace, 2008). This time-dependent change demonstrates that with acute exercise, FMD is biphasic (Dawson et al., 2013). Interestingly, active men who were overweight had an enhanced endothelial response following acute aerobic exercise, however the inactive over-weight men had an attenuated response (Harris et al., 2008). Between the inactive and active overweight groups there was a disparity in CRP, blood pressure (BP), and triglycerides (Harris et al., 2008). In addition to acute exercise increasing FMD, there is evidence that acute exercise can reduce triglycerides, reduce BP, increase high-density lipoprotein cholesterol (HDL), improve insulin sensitivity, and improve glucose homeostasis (Thompson et al., 2001). This suggests acute bouts of exercise are beneficial for anti-atherogenic effects (Harris et al., 2008). In a study with obese women that participated in circuit training for 8 weeks, Franklin et al. (2015) demonstrated that endothelial function following a single bout of strenuous weightlifting improved, indicating that training status, type, and duration of exercise can influence endothelial function.

B. High-intensity Interval Exercise

High-intensity interval exercise training (HIIE training, chronic) or high-intensity interval exercise (HIIE, acute) have become popular because individuals can train at very low volumes (time) while maintaining a high work output (Gibala & McGee, 2008). Additionally, following 6 HIIE training sessions in just 2 weeks, muscle oxidative potential and endurance capacity have been shown to improve (Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005). The popularity of this type of training has also made its way to clinical populations, as such, aerobic HIIE training have been shown to

be superior to moderate intensity exercise for increasing endurance capacity in patients with coronary artery disease in just 10 weeks (Rognmo et al., 2004). Aerobic interval training (AIT) protocols typically used 4 minutes of high-intensity work (80-95% VO_{2max}) followed by 3-4 minutes of recovery time, with 4-6 cycles either on a cycle ergometer or treadmill (Peter S Munk, Staal, Butt, Isaksen, & Larsen, 2009; Rognmo et al., 2004; Warburton et al., 2005; Wisløff et al., 2007). These studies' protocols have been shown to be acceptable for patients with coronary heart disease, heart failure, and other cardiac patients following stent implantation (Peter S Munk et al., 2009; Rognmo et al., 2004; Warburton et al., 2005; Wisløff et al., 2007). Importantly, 8-weeks of HIIE training have been shown to be beneficial for the overweight and obese population by improving aerobic capacity and by decreasing fat mass (Wallman et al., 2009). A single bout of HIIE in patients with coronary heart disease have been shown to be safe with no negative consequences (Guiraud et al., 2011). Interestingly, HIIE have been shown to reduce endothelial dysfunction when compared to continuous moderate-intensity exercise (CME) following a high-fat meal (Tyldum et al., 2009). Tyldum et al. (2009) discussed that HIIE increased and maintained higher total antioxidant status helping to blunt endothelial dysfunction. Suggesting that NO bioavailability can be increased by HIIE with the associated increase in antioxidant status (Tyldum et al., 2009). A recent study by Sawyer et al. (2016) has demonstrated HIIE training but not CME training improved FMD in obese adults, however the CME training increased the resting brachial artery diameter and low flow-mediated constriction (L-FMC) indicative of improved vascular health. Finally, a review and meta-analysis by Ramos et al. (2015) concluded HIIE training improves FMD 2.26% more than CME training.

The Role of C1q-TNF-related protein-9 on Vasodilation

C1q-TNF-related protein-9 (CTRP9) is a newly discovered adipokine, a part of the C1q-TNF-related proteins (CTRPs) family of adiponectin paralogs (Davis & Scherer, 2008; Guang W Wong, Wang, Hug, Tsao, & Lodish, 2004). However, CTRP9 shares the greatest amino acid overlap with adiponectin (G. William Wong et al., 2009). Total plasma CTRP9 has been shown to be reduced in diabetic animals (Su et al., 2013). Additionally, CTRP9 deficient mice were obese and insulin resistant and developed hepatic steatosis as well as reduced skeletal muscle AMPK activation and mitochondrial content (Yi et al., 2011). Another study demonstrated that CTRP9 attenuates hepatic steatosis via the autophagy-mediated inhibition of endoplasmic reticulum stress (T. W. Jung et al., 2015), suggesting genetic evidence for a physiological role of CTRP9 in cardiovascular homeostasis (Yuan et al., 2015). Furthermore, CTRP9 has been shown to increase eNOS activation via the AMPK-Akt-eNos mechanism in human umbilical vein endothelial cells (Zheng et al., 2011). A study by Jung et al. (2016) demonstrated that CTRP9 attenuates cytokine-induced vascular inflammation in endothelial cells mediated by AMPK activation. Moreover, treatment with CTRP9 decreased TNFa-induced activation of NF-kB, adhesion molecules (ICAM-1 and VCAM-1), as well as monocyte chemoattractant protein-1 (C. H. Jung et al., 2016). Additionally, CTRP9 overexpression in pulmonary artery epithelial cells increased eNOS and reduced the secretion of ET-1 (Li, Geng, Wang, Cheng, & Xu, 2016). Importantly, other studies have shown that CTRP9 is down-regulated in obese mice (Uemura et al., 2013) and patients with insulin resistance (Hwang et al., 2014; Wang et al., 2015). Furthermore, serum CTRP9 is inversely correlated with visceral fat in humans (Hwang et al., 2014). Taken together,

these findings suggest the important role of CTRP9 on the modulation of endothelial function, especially in obese individuals. However, a recent study suggests that CTRP9 levels are elevated in obesity and significantly decrease following weight loss surgery (Wolf et al., 2016). Wolf et al. (2016) suggests CTRP9 may play a compensatory role in obesity, such as insulin, illustrating a need for further studies with CTRP9 in obese populations.

Conclusion

As obesity's prevalence continues to increase, there is a growing need to understand underlying mechanisms of treatments to mediate the many negative consequences of obesity. Cardiovascular disease is among the leading causes of morbidity and mortality, and is associated with obesity (N. B. Johnson et al., 2014). Again, the impairment of endothelial function is the earliest detection for cardiovascular disease (Singhal, 2005). Therefore, biomarkers that regulate endothelial and vascular function can be useful to monitor the progress of interventions used to combat these diseases. As such, there is limited information regarding the association of exercise-mediated CTRP9 with endothelial function. Thus, this study investigated whether CTRP9 could be a reliable biomarker to predict outcomes of exercise treatments in obesity, with a focus on HIIE compared to CME. Additionally, this study aimed to determine if CTRP9 is correlated with endothelial function (FMD), as well as investigate its relationship with obesity.

CHAPTER III: METHODS

Subjects

Sixteen (9 obese and 7 normal-weight) healthy male subjects 18-45 years old were recruited to participate in the study. Subjects with a body mass index (BMI) above 30 kg/m^2 were classified as obese, and those with a BMI between 18.5 and 24.9 kg/m^2 were classified as normal-weight. All subjects completed an informed consent form, a medical history questionnaire, and 7-day physical activity record prior to data collection. The study was approved by the Florida Atlantic University's Institutional Review Board.

Subjects were excluded from the study if they had any known or suspected cardiovascular, metabolic, rheumatologic, or other inflammatory disease. Subjects were also excluded from the study if they were taking any medication or supplements, users of tobacco products (cigarettes, cigars, chewing tobacco, vapors), or if they consumed an average of more than ten alcoholic beverages per week. These exclusion criteria were determined using the health history questionnaire. Subjects fasted overnight for at least eight hours and abstained from alcohol, caffeine intake, and intense physical activity for at least 24 hours prior to each lab visit.

Experimental Protocol

All subjects performed three exercise protocols, with a minimum of one week transpiring between each session. Subjects arrived at the Exercise Biochemistry Laboratory between 7:00-7:30AM. During the first visit, following completion of the informed consent form, medical history questionnaire, and 7-day physical activity

questionnaire, height and weight was measured (SECA 769, Chino, CA) along with hip and waist circumference. Additionally, following 20 minutes of sitting, resting heart rate was recorded using a heart rate monitor (Polar T31, Polar Electro, Kempele, Finland) and blood pressure was measured using a sphygmomanometer (752M-Mobile Series, American Diagnostic Corporation, Hauppauge, NY). Immediately following the assessments above, blood sampling was performed by a trained phlebotomist using standard aseptic techniques. A closed IV catheter system (BD Nexiva 20GA, REF 383516, Franklin Lakes, NJ, USA) was inserted in the superficial vein of the upper arm. Approximately two tablespoons of blood volume (30mL) were collected into specific collection tubes for subsequent analysis. Prior to the beginning of the exercise protocol, subjects lay supine on the ultrasound bench for 20 minutes before the assessment of their resting FMD measurement using an ultrasound (Phillips iU22, Foster City, CA, USA).

Subjects participated in a graded exercise test (approximately 12 to 15 minutes) on a treadmill (Norditrack X11i) designed to assess maximal oxygen consumption ($VO_{2\ max}$) measured by open-circuit spirometry (ParvoMedics Metabolic Measurement System (ParvoMedics, Sandy, UT, USA)) and maximal heart rate (HR_{max}). The maximal exercise protocol began with a three-minute warm up at 60% age-predicated maximal heart rate (HR_{max}), followed by an increase in speed until 80% HR_{max}. Subsequently, the grade was increased by 2% every two minutes until attainment of VO_{2max}. The validation of VO_{2max} was determined by either the primary criterion of a plateau in VO_2 or 2 of the 3 secondary criteria are achieved. The secondary criteria are (1) reaching predicted maximal heart rate, (2) achieving a respiratory exchange ratio of >1.15, and (3) reporting a rating of perceived exertion (15-point Borg Scale) of 19 or 20. Importantly, this

perceived exertion scale was used to measure the perception of stress each minute during exercise. If subjects reported a difficulty to maintain exercise intensity, then the exercise testing was terminated. Two tablespoons of blood volume were collected along with FMD measurements upon immediate completion of the exercise protocol, 1 hour, and 2 hours into recovery.

During the second and third visits, subjects were randomly assigned to participate in either HIIE or CME following a previously published treadmill protocol (Rognmo et al., 2004; Tyldum et al., 2009). The HIIE consisted of 30-minutes of exercise, including a 5-minute warm-up period of 65% – 75% HR_{max}, 4 intervals of 4 minutes at an intensity that elicits 85% - 95% HR_{max}. Between the intervals the subjects walked for 3-minutes at 65% - 75% HR_{max}. To equate the total work (caloric expenditure) conducted by both exercise protocols, CME consisted of 38 minutes at 65% - 75% of HR_{max}. Blood collection and FMD measurements for both visits 2 and 3, followed the same protocol as the first visit.

Blood Sampling

During each blood draw, 5mL of blood volume was collected into a serum separation tube (SST) for serum protein analysis and centrifuged for 10 minutes at 1300 × g at room temperature. The serum was collected and stored in aliquots at –80° C for subsequent analysis by enzyme-linked immunosorbent assays (ELISA). Serum CTRP9 was analyzed using (Cloud-Clone Corp. Houston, TX, USA).

FMD Measurement

The FMD measurement and protocol followed established guidelines (Harris, Nishiyama, Wray, & Richardson, 2010). The brachial artery was identified and imaged

longitudinally using a Phillips L9-3 broadband 9.0 MHz vascular probe on the medial upper arm 2–10 cm above the antecubital fossa near the level of the heart. Landmarks were identified to ensure the same location for all repeated FMD measurements. Diameter and blood flow velocity was recorded in Digital Imaging and Communications in Medicine (DICOM) format using a duplex mode of ultrasound that allows simultaneous B-mode imaging for diameter measurements and Doppler for blood velocity (shear rate) using a Philips iU22 ultrasound. The insonation angle was set to 60° and gate width was adjusted for an accurate measurement of blood flow as described by Harris et al. (2010).

Subjects laid supine for 20 minutes prior to baseline FMD measurements, following acclimatization baseline measurements were recorded for 1 minute. A blood pressure cuff (WelchAllyn 406920 series) was placed two centimeters distal to the antecubital fold and was then be inflated to ≤ 250 mmHg for 5 minutes. Prior to the cuff release, measurements were recorded for 20 seconds, following release post-occlusion measurements were recorded for 3 minutes.

Validated software (Medical Imaging Applications, LLC.) was used for offline analysis of the recorded images for brachial diameter (mm) and blood velocity (shear rate). ECG gating was used for consistent cardiac cycles (end diastole) for brachial diameter measurements. FMD (%) was quantified as the peak diameter observed post-occlusion and reported as percent change from the average 1-minute baseline diameter.

Statistical Analyses

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS version 22.0). Differences between obese and normal-weight groups in baseline

variables were computed by independent t tests. A 2 (group) × 2 (treatment) × 4 (time points: Pre, Post, R1, and R2) repeated measures analyses of variance (ANOVA) were utilized to examine the effect of acute aerobic exercise on serum levels of CTRP9 and FMD. The Greenhouse-Geisser correction of degrees of freedom was used when sphericity assumptions were violated. Pearson product-moment correlations were used to examine the relationship of CTRP9 with FMD. Statistical significance was defined as a P-value ≤ 0.05.

CHAPTER IV: RESULTS

Anthropometric Measurements of the Study Participants

As shown in Table 1, the analysis revealed a significant difference in age, weight, BMI, relative VO_{2max}, waist circumference, hip circumference, waist-to-hip ratio, resting systolic blood pressure, and diastolic blood pressure between obese and normal-weight subjects.

Table 1: Subject Anthropometric Characteristics

Variables	Normal-weight (N=7)	Obese (N=9)	P Value
Age	22.77 (± 1.63)	27.53 (± 4.81)	0.019*
Height (m)	1.79 (± 0.04)	1.78 (± 0.06)	0.815
Weight (kg)	71.51 (± 10.03)	116.41 (± 17.60)	< 0.001*
BMI (kg/m^2)	22.21 (± 2.00)	36.41 (± 3.92)	< 0.001*
Waist (cm)	80.04 (± 7.35)	113.14 (± 15.94)	< 0.001*
Hip (cm)	95.50 (± 5.10)	117.94 (± 8.03)	< 0.001*
WHR	0.837 (± 0.06)	0.953 (± 0.09)	0.009*
rHR	66.57 (± 10.36)	73.44 (± 5.45)	0.148
rSBP	115.57 (± 6.90)	138.11 (± 11.58)	< 0.001*
rDBP	71.63 (± 4.83)	84.66(± 7.63)	0.001*

Note: Data are represented as mean ± SD. BMI = body mass index; WHR = waist-to-hip ratio; rHR = resting heart rate; rSBP = resting systolic blood pressure; rDBP = resting diastolic blood pressure.

Measurement of Serum CTRP9 and Flow-Mediated Dilation

At baseline, no difference was observed in CTRP9 and FMD between obese and normal-weight subjects. Repeated measures ANOVA demonstrated a significant time effect for CTRP9 immediately following acute HIIE and CME in both groups ($F_{[3, 42]} = 5.435$, $p = 0.003$) (see Figure 1). Furthermore, both significant treatment by time and group by time interactions for FMD were observed following both exercise protocols ($F_{[3, 42]} = 3.728$, $p = 0.018$; $F_{[3, 42]} = 4.346$, $p = 0.009$; respectively), with a greater CME-induced FMD response at two hours into recovery in obese subjects than normal-weight subjects (see Figure 2).

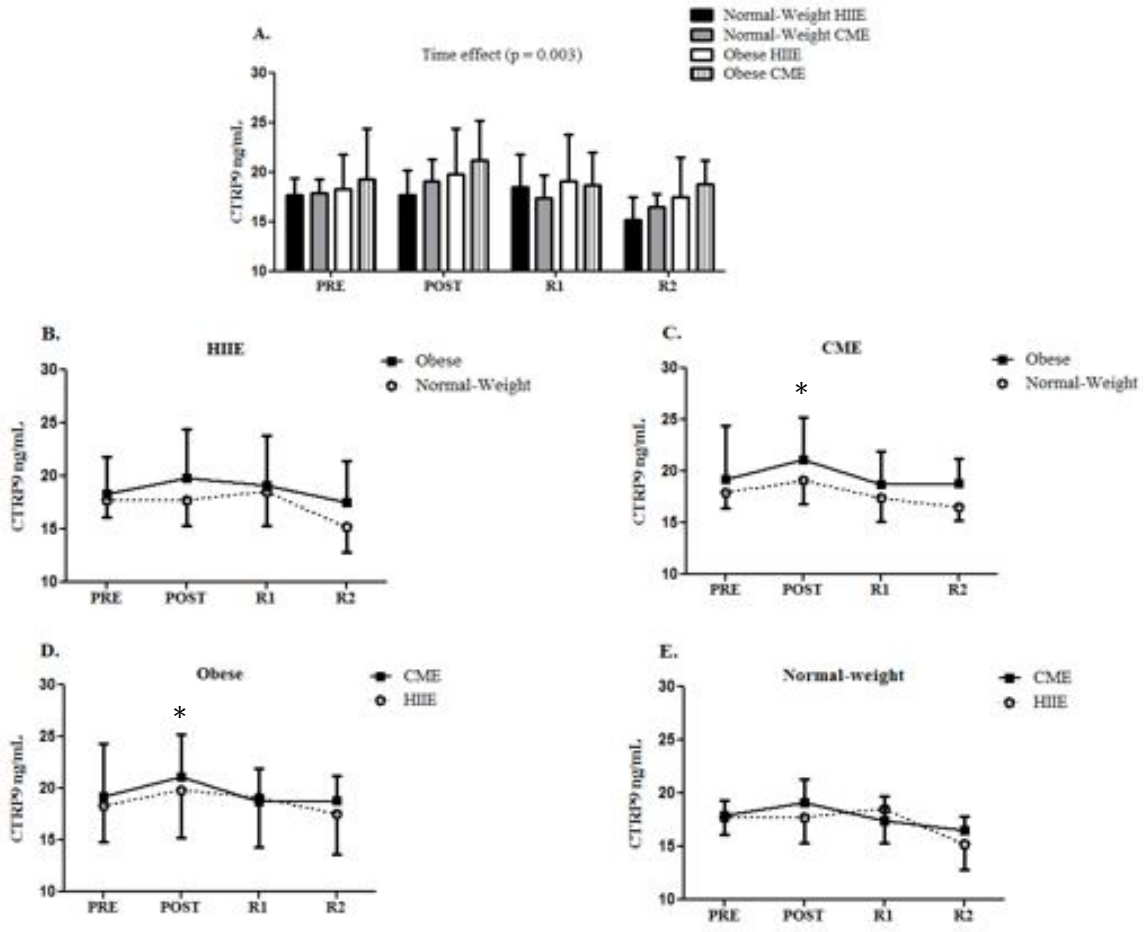

Figure 1. The response of serum CTRP9 following HIIE vs. CME. Data are presented as means ± SD.

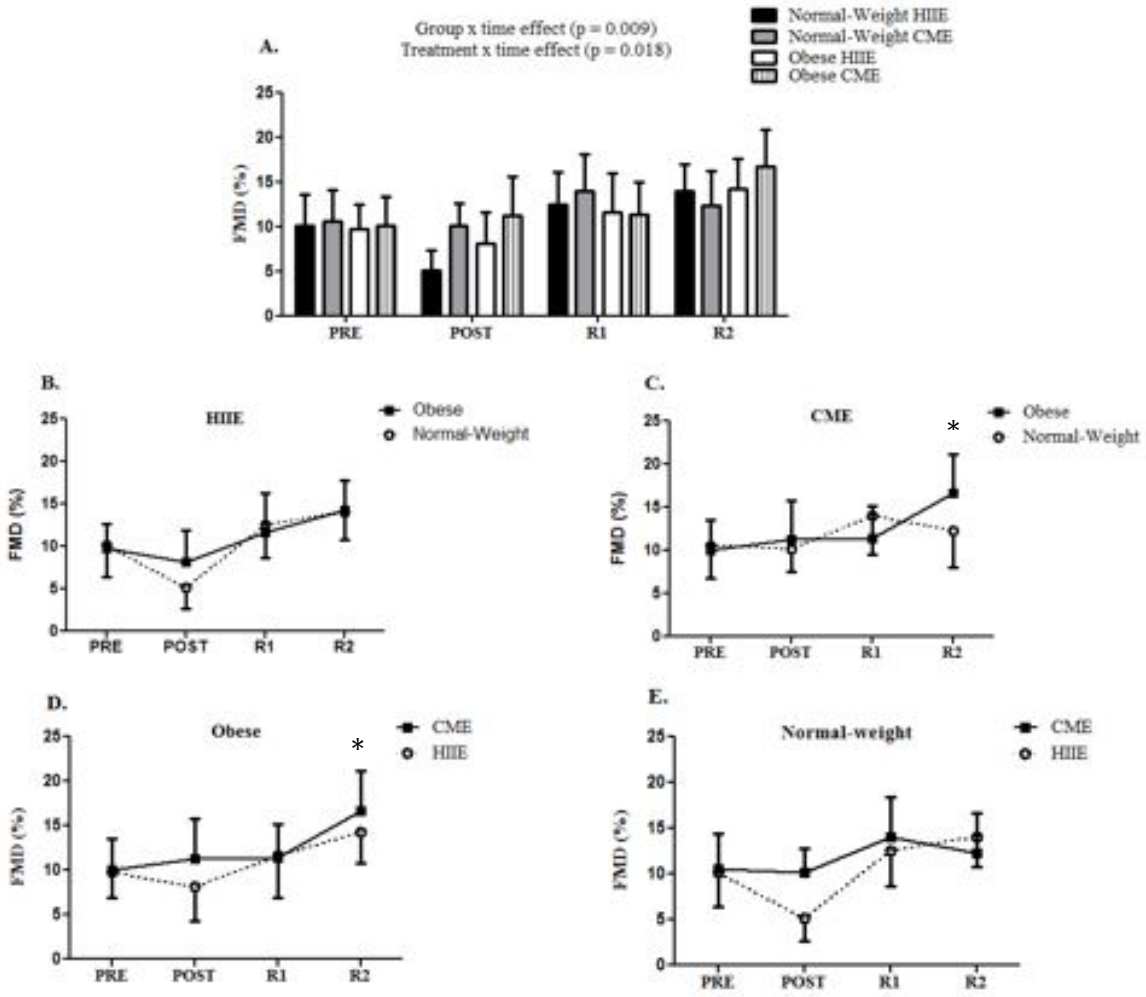

Figure 2. The FMD response to HIIE vs. CME. Data are presented as means ± SD.

Correlations Between CTRP9 and FMD

At baseline, our analyses did not observe a significant correlation between CTRP9 and FMD. Moreover, a positive correlation in percent change (baseline to peak value) between CTRP9 and FMD was found following acute CME ($r = 0.589$, $p = 0.016$) (see Figure 3B). However, this relationship between CTRP9 and FMD following HIIE failed to exist ($r = -0.206$, $p = 0.444$) (see Figure 3A).

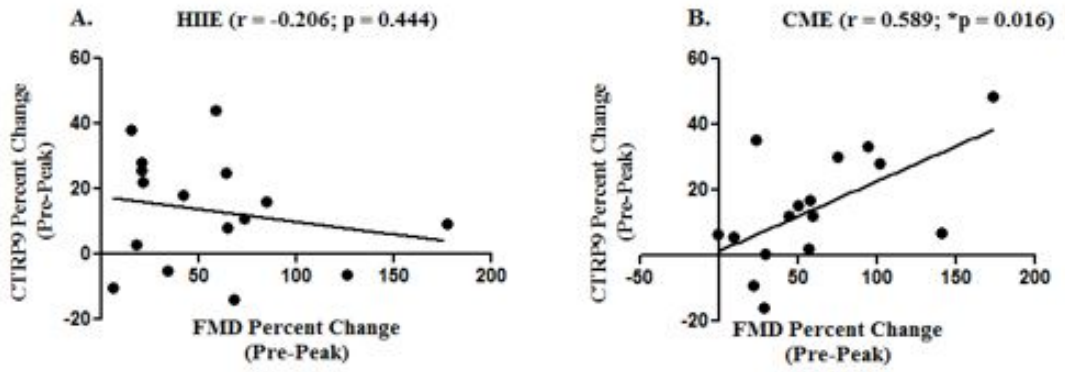

Figure 3. The relationship between CTRP9 percent change (pre-peak value) and FMD percent change (pre-peak value).

CHAPTER V: DISCUSSION

This study examined the effect of acute HIIE vs. CME on serum CTRP9 and brachial artery FMD responses in obese and normal-weight subjects. Our results demonstrated that obese subjects elicited a similar elevation in serum CTRP9 immediately following both acute HIIE and CME compared to normal-weight subjects. Furthermore, this study also showed both treatment and group effects across time in FMD with a greater elevation in obese subjects following acute CME. These findings support that CME is an effective modality to improve cardiovascular health in obese individuals.

While this study did not observe a difference at the baseline level of CTRP9 between obese and normal-weight subjects, the literature regarding CTRP9 and obesity remains controversial. Specifically, Peterson et al. (2013) found that circulating levels of CTRP9 are lower in obese mice following a high-fat diet than controls (lean mice). Further research utilized a CTRP9 knockout mice model and demonstrated that mice became obese even when fed normal chow (Wei, Lei, Petersen, Aja, & Wong, 2014). In humans, CTRP9 is inversely related to visceral fat (Hwang et al., 2014). In contrast, Wolf et al. (2016) found serum levels of CTRP9 were higher in obese than normal-weight individuals, but a decrease in CTRP9 was observed following weight-loss surgery. While lower serum CTRP9 levels in metabolically unhealthy individuals were observed (Hwang et al., 2014), participants in this study did not have a history of metabolic syndrome or diseases, which eliminated disease-related confounding factors when comparing their resting level of CTRP9 to normal-weight counterparts.

To the best of our knowledge, this study is the first to demonstrate that acute exercise (HIIE or CME) can up-regulate the expression of serum CTRP9 in either obese or normal-weight individuals. The literature has reported that CTRP9 plays a compensatory role in obesity and arterial stiffness (C. H. Jung et al., 2014; Wolf et al., 2016). For example, CTRP9 promotes vasodilation through eNOS activation via the AMPK-Akt-eNos mechanism in human umbilical vein endothelial cells (Zheng et al., 2011). Furthermore, the expression of increased CTRP9 in pulmonary epithelial cells enhanced the activation of eNOS and reduced the release of vasoconstricting factor, such as endothelin-1 (Li et al., 2016). Although there is limited information regarding exercise-mediated CTRP9, research has previously shown that both acute HIIE and CME improve indicators of endothelial function (e.g., total nitric oxide) (Chuensiri, Tanaka, & Suksom, 2015; Currie et al., 2012; Harris et al., 2008; Kaya et al., 2009). Additionally, a recent study by Jung et al. (2016) has demonstrated that CTRP9 attenuates cytokine-induced vascular inflammation in endothelial cells mediated by AMPK activation, specifically by decreasing TNF-α induced activation of inflammatory transcription factor (NF-kB) and adhesion molecules (ICAM-1 and VCAM-1). Taken together, these findings suggest exercise-induced CTRP9 may potentially play a role in the improvement of cardiovascular health and modulation of obesity-associated metabolic complication, such as insulin resistance (Hwang et al., 2014; Wolf et al., 2016).

The measurement of FMD has been shown to provide prognostic information that may exceed the assessment of traditional risk factors for cardiovascular event (Green, Jones, Thijssen, Cable, & Atkinson, 2011). Although FMD has been demonstrated to be impaired in obese individuals (Davison et al., 2011), the present study did not observe

any difference at baseline with normal-weight individuals. However, Davison and colleagues utilized older participants, which may account for obesity-related difference in FMD. Importantly, this study demonstrated significant group by time and exercise mode by time effects in FMD, with a greater CME-induced response in obese than normal-weight subjects, which remained elevated two hours into recovery. These findings may support the modality of acute CME in the improvement of endothelial function in obese population (Zhu et al., 2010). Specifically, enhanced FMD response following acute CME may be a result of the exercise-mediated shear stress on the endothelium to enhance NO bioavailability (Zhu et al., 2010). Although this study is the first to examine the impact of obesity on FMD response following acute HIIE vs. CME, acute HIIE has been demonstrated to improve FMD similarly when compared to CME in patients with coronary artery disease (Currie et al., 2012). However, Currie et al. (2012) reported a greater total workload in acute CME than HIIE while this study equated total work (caloric expenditure) between exercise conditions (HIIE and CME) in both groups, which may potentially explain the variance in these findings. Finally, a significant positive relationship in percent change (baseline to peak value) between CTRP9 and FMD was found following acute CME, providing evidence of enhanced endothelial function in obese individuals (Zhu et al., 2010). However, additional investigation is needed to further verify the relationship of exercise-mediated CTRP9 and endothelial function in obesity.

In conclusion, this study demonstrated that obese subjects exhibited a similar CTRP9 response following both acute HIIE and CME compared to normal-weight subjects. Furthermore, obese subjects exhibited a greater FMD elevation in response to

CME compared to normal-weight subjects. While HIIE is a time-effective strategy to improve metabolic complications and systemic inflammation (P. S. Munk et al., 2011; Shiraev & Barclay, 2012), the novel results from this study provide a foundation for additional examination of the mechanisms of exercise-mediated CTRP9 on endothelial function in obese individuals. Finally, the measurement of CTRP9 could potentially predict the effectiveness of exercise treatments to prevent or delay obesity-associated cardiovascular events.

APPENDICES

APPENDIX A: Medical History Form

Medical History Questionnaire

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

Part I: Subject Information

_____ Name (Print)

_____ Home Phone

_____ Current Mailing Address

_____ Work Phone

_____ Personal Physician

_____ Email Address

_____ Person to Contact in Case of Emergency

_____ Phone

Gender: _____Female _____Male Date of Birth: _____

If female, the menstrual cycle is: _____follicular phase_____ ovulatory phase_____ luteal phase

Height: _____ cm Weight: _____kg

Resting Blood Pressure: Systolic BP_____/Diastolic BP_____

Resting heart Rate: _____beats/min

Waist circumference: _____cm

Hip circumference: _____cm

Part II. Medical 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 problem/Murmur		

Have you been diagnosed with an autoimmune disease? If yes, please circle the appropriate disease.

Rheumatoid arthritis Lupus	Crohn's Disease
Type I Diabetes	Type II Diabetes
Multiple Sclerosis Psoriasis	Other _____ (please specify)

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

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

Have you ever been diagnosed with a psychological disorder? _____Yes _____No

Part III. Health Related Behavior

Do you smoke? _____Yes _____No

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 minutes, 3 times per week)? _____Yes _____ No

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

How many minutes of exercise do you do per week? _____

Have you recently (within 1 month) experienced a major negative life event (i.e., death in family, divorce)? _____Yes _____No

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

If yes, please list: _____

APPENDIX B: 7-day Physical Activity Questionnaire

This questionnaire is called the Seven-Day Physical Activity Recall. The information from it will be used to estimate the number of calories you burn up through physical activity.

1. On average, how many hours did you sleep each night during the last five weekday nights, Sunday through Thursday?

Enter a numeric value (0 if not applicable) ___ ___. ___

2. On average, how many hours did you sleep the last Friday and Saturday nights?

Enter a numeric value (0 if not applicable) ___ ___. ___

3. How many hours did you spend during the last five weekdays doing these moderate activities or others like them?

Enter a numeric value (0 if not applicable) ___ ___. ___

4. How many hours did you spend last Saturday and Sunday doing these moderate activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

5. How many hours did you spend during the last five weekdays doing these hard activities or others like them?

Enter a numeric value (0 if not applicable) ___ ___. ___

6. How many hours did you spend last Saturday and Sunday doing these hard activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

7. How many hours did you spend the last five weekdays doing these very hard activities, or others like them?

Enter a numeric value (0 if not applicable) ___ ___. ___

8. How many hours did you spend last Saturday and Sunday doing these very hard activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

9. Were you employed outside the home during the last seven days? Of no, put zeros for questions 9-13. If yes, how many days?

Enter a numeric value (0 if not applicable) ___ ___. ___

10. How many hours per day?

Enter a numeric value (0 if not applicable) ___ ___. ___

11. How many of these hours per day were spend doing moderate activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

12. How many of these hours per day were spent doing hard activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

13. How many of these hours per day were spent doing very hard activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

14. Compared to your physical activity over the past three months, was last week's physical activity more, less, or about the same?

1- More 2- Less 3- About the same

Very Hard Activities (>7.0 METs)

These include strenuous sports involving a lot of movement and running. Very few household or occupational tasks are included, except carrying heavy loads, digging or chopping with heavy tools, or other similar hard physical labor.

- Boxing – in ring, sparring
- Circuit training
- Climbing hills with 5-20kg load
- Cycling, racing (intensive)
- Digging ditches
- Farming – barn cleaning
- Field Hockey
- Football
- Forestry – fast ax chopping, barking trees, carrying logs, sawing by hand
- Gardening, digging
- Marching, rapid
- Racquetball
- Rope jumping
- Running, jogging – cross country, 6-10 min/mile
- Skiing, cross country
- Skin diving as frogman, moderate motion
- Soccer
- Squash
- Swimming, continuous – intensive
- Tennis, singles

Moderate Activities (3-5 METs)	Hard Activities (5.1-6.9 METs)
These activities involve modest increases in heart rate and breathing – e.g., many household and home repair tasks • Bowling • Calisthenics without weights • Carpentry • Childcare • Cleaning (vacuuming, sweeping) • Croquet • Cycling – leisure, 5.5 mph mild • Electrical work • Feeding farm animals • Fencing • Frisbee playing • Gardening – hedging, planting, mowing • Golf – no power cart • Grocery shopping • Gymnastics • Cooking • Horse shoes • Horseback riding • Laundry • Locksmith • Mopping floor • Motor-cross • Music – playing drums • Painting – outside • Plastering • Sailing • Surfing • Sweeping • Swimming – mild • Table tennis • Tai-chi • Window cleaning • Yoga	Most people will have noticeable increases in breathing and will likely perspire – e.g., vigorous household, home repair, and gardening tasks, heavy industrial work, and some construction and vigorous sports • Aerobic dance • Badminton • Climbing hills with no load • Coal shoveling • Cycling – leisure, 9.4 mph (moderate) • Farming – shoveling grain • Fast walking • Folk dancing • Forestry – hoeing, planting by hand • Karate or Judo • Roller skating • Scrubbing floors • Skiing, water or downhill • Tennis, doubles • Walking on level brisk or striding • Weight lifting or training • Swimming, moderate

APPENDIX C: Participant Consent Form

ADULT CONSENT FORM

Consent Form Version & Date: Version 3.0; May 20 2016

1) Title of Research Study: Exercise-mediated C1q/TNF-Related Protein-9 and Flow-Mediated Vasodilation in Obesity

2) Investigator(s): Chun-Jung Huang, Ph.D., Brandon Fico, B.S.

3) Purpose: The purpose of this study is to understand how exercise may regulate vasodilation.

4) Procedures: If you decide to be in this research study, you will be asked to fast overnight for at least eight hours and to abstain from alcohol, caffeine intake, and intense physical activity for at least 24 hours before attending three laboratory visits separated by at least one week. It will take approximately 4 hours for each visit. You will be asked to arrive at the Exercise Science Biochemistry Laboratory between 7:00 AM and 7:30 AM on the Boca Raton Campus. During session one, following completion of the informed consent form, medical history questionnaire, and 7-day physical activity questionnaire, your height and weight will be obtained with a standard medical device. Additionally, your resting heart rate will be assessed by a heart rate monitor. Blood pressure will be measured using a sphygmomanometer, and your hip and waist circumference will be assessed by a tape measure. All assessments will be performed by a research assistant.

Immediately following the assessments above, blood sampling will be performed by a trained phlebotomist using standard aseptic techniques. A closed IV catheter system (20 gauges) will be inserted in the superficial vein of the upper arm. Two tablespoons of blood volume (30 mL) will be collected into specific collection tubes for subsequent analysis. Prior to the beginning of exercise protocol, subjects will be lying with face up on the treatment bed for 20 minutes before their testing vascular function measurement using an ultrasound (a technique to measure the size of vessel and blood flow) with a trained technician. A blood pressure cuff will be placed two centimeters (approximately 0.8 inch) away from the antecubital fold and increased to ≤ 250 mmHg for 5 minutes. You will then participate in a graded running exercise test on a treadmill designed to assess maximal oxygen consumption (VO_{2max}). After exercise, heart rate and blood pressure will be assessed, and two tablespoons of blood volume will be collected as well as vascular function measurements upon immediate completion of the exercise protocol, 1 hour, and 2 hours into recovery.

During the second and third visits, you will randomly be assigned to participate in either continue moderate-intensity exercise (CME) and high-intensity interval exercise (HIIE) on a treadmill. The HIIE consists of 30-minutes of exercise, including a 5-minute warm-up period of 50% - 60% of VO_{2max}, 4 intervals of 4 minutes at an intensity that elicits 80% - 90% of VO_{2max}. Between the intervals the subjects will walk for 3-minutes at 50% - 60% VO_{2max}. To equate the total work perform by both exercise protocols, CME consists of 38 minutes at 50% - 60% VO_{2max}. Blood collection and vascular function measurements for both visits 2 and 3, will follow the same protocol as the first visit. However, you will be excluded from the study if you have known or suspected cardiovascular, metabolic, rheumatologic,

Participant Initials

or other inflammatory diseases/conditions. You will be also excluded from the study if you are taking
any medication and supplements, users of tobacco products (cigarettes, cigars, chewing tobacco), or if you consume an average of more than ten alcoholic beverages per week. Finally, if you are pregnant or nursing, you will be also excluded from the study because of the potential effects on immune responses.

5) Risks: Since no drugs will be given to you in this study, there is no risk of you having a reaction to any of the substances that are being studied. However, inserting the needle and drawing blood from your arm may cause pain, bruising lightheadedness, fainting, and on rare occasions, infection. There may be some slight discoloration and a bruise at the site of the needle insertion. To minimize these risks, a trained phlebotomy technician will perform all blood sampling. While the total amount of blood drawn is small compared to the amount taken when you donate blood, this will minimize the risk of fainting. Since you will perform aerobic exercise, the leg soreness caused by muscle damage may be experienced within 24 to 48 hours. The muscle soreness should be eased after 48 or 72 hours. Other potential risks, including fainting, a temporary dizziness, or falling off the treadmill may also be associated with exercise, and the instructions for these cares will be provided to you. In an unexpected event (e.g., unusual blood pressure response, arrhythmia, heart attack) associated with exercise that necessitate emergency care, the investigator(s) will initiate activate the Exercise Biochemistry Laboratory emergency procedures, such as automated external defibrillator (AED). Research staff who are trained in CPR will activate the emergency medical system (i.e. call 911) in addition to being equipped with a defibrillator.

6) Benefits: Data collected during this study will contribute to general scientific knowledge regarding how exercise may regulate vasodilation. You will receive free assessments of hip/waist circumference, blood pressure, heart rate, and maximal oxygen consumption (VO_{2max}).

7) Data Collection & Storage: Potentially identifiable information about you will consist of a medical history questionnaire and research data sheets. The blood samples will be stored in the freezer in the Exercise Science Laboratory and be discarded into biohazard waste containers within 5 years after completion of the study. Data are being collected only for research purposes. All personal identifying information will be kept in password protected files. The key linking your name to your data will be deleted within 2 years after completion of the study. De-identified data records will be kept in a locked file cabinet in the Dr. Huang's office. Although results of this research may be presented at meetings or in publications, identifiable personal information pertaining to participants will not be disclosed.

8) Data Registries: I give permission for my data and blood samples to be stored and used to answer future scientifically responsible research questions about the effect of exercise on certain biological indicators of inflammation and metabolism. If at some time in the future you change your mind about future research, please contact Dr. Huang at the address below. Please check YES or NO below.

YES_____ NO_____

Participant Initials

_____

9) Contact Information:

- If you have questions about the study, you should call or email the investigator(s) Mr. Brandon Fico at (954) 662-4318 / ***bfico@my.fau.edu*** or Chun-Jung Huang at (561) 561-2971 / ***chuang5@fau.edu***
- If you have questions or concerns about your rights as a research participant, contact the Florida Atlantic University Division of Research at (561) 297-0777 or send an email to researchintegrity@fau.edu.

10) Consent Statement:

*I have read or had read to me the information describing this study. All my questions have been answered to my satisfaction. I am 18 years of age or older and freely consent to participate. I understand that I am free to withdraw from the study at any time without penalty. I have received a copy of this consent form.

I agree _____ I do not agree _____ be audiotaped/videotaped.

Printed Name of Participant: _____

Signature of Participant: _____ Date: _____

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