EFFECTS OF A SIX-WEEK WATERMELON SUPPLEMENTATION ON INSULIN RESISTANCE AND FOOD INTAKE SIGNALING IN FREE-LIVING, OVERWEIGHT, POST-MENOPAUSAL WOMEN

A Thesis
by
THOMAS JACOB JURRISSEN

Submitted to the Graduate School
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

May 2015
Department of Health and Exercise Science

EFFECTS OF A SIX-WEEK WATERMELON SUPPLEMENTATION ON INSULIN RESISTANCE AND FOOD INTAKE SIGNALING IN FREE-LIVING, OVERWEIGHT, POST-MENOPAUSAL WOMEN

A Thesis
by
THOMAS JACOB JURRISSEN
May 2015

APPROVED BY:

Jennifer J. Zwetsloot, Ph.D.
Chairperson, Thesis Committee

Kevin A. Zwetsloot, Ph.D.
Member, Thesis Committee

R. Andrew Shanely, Ph.D.
Member, Thesis Committee

N. Travis Triplett, Ph.D.
Chairperson, Department of Health and Exercise Science

Max C. Poole, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

Abstract

EFFECTS OF A SIX-WEEK WATERMELON SUPPLEMENTATION ON INSULIN RESISTANCE AND FOOD INTAKE SIGNALING IN FREE-LIVING, OVERWEIGHT, POST-MENOPAUSAL WOMEN

Thomas Jacob Jurrissen
B.S., Appalachian State University
M.S., Appalachian State University

Chairperson: Jennifer J. Zwetsloot, Ph. D.

Obesity has been associated with hormonal disruption involving food intake and utilization, and post-menopausal women have a higher propensity to be overweight. PURPOSE: Investigate the effects of six-weeks of watermelon supplementation on insulin resistance and food intake signaling in free-living, overweight, post-menopausal women. METHODS: Subjects (60.1±6.8 years old and 59.5±5.2 years old, control and watermelon, respectively throughout) were overweight (82.5±14.5 kg and 84.2±17.1 kg, respectively) and were randomly assigned to either the watermelon or control treatment groups. The watermelon group consumed 710 mL of watermelon puree per day for six weeks. No supplement was provided to the control group, i.e., the control group served as a time control. Both groups were asked to maintain their lifestyle and to not intentionally lose body mass. Fasting blood was collected pre- and post-six-week supplementation period. RESULTS: Watermelon supplementation increased L-arginine ($p<0.05$), but did not change glucose, insulin, or Homeostatic model assessment of insulin resistance ($p>0.05$). The hunger and

satiety peptides also did not change as a result of watermelon supplementation: Ghrelin, leptin, glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1, or peptide YY ($p>0.05$). The control group, however, showed increased levels of PYY ($p<0.05$). There was also a main effect of time for leptin, insulin, and GLP-1 ($p<0.05$). CONCLUSIONS: Six-weeks of watermelon supplementation did not impact insulin resistance or food intake signaling in free-living, overweight, post-menopausal women. The community-based design of this study may account for the variability observed within the control group.

Acknowledgements

I would like to thank my committee members Jennifer Zwetsloot, Kevin Zwetsloot and Andrew Shanely for all their time and effort throughout the present study. I appreciate all the hard work they have put into this project and into helping me become a better scientist, writer and student. I would also like to thank my fellow graduate students, Shaun Woerner, Juliane Young, and Lauren Carson for all their hard work in assisting with the present study. I would also like to thank the assistance from the undergraduate students, Chase Sherman, Nicholas Charron, Anna Bishop, and Emma Elsea for all their hard work and dedication. I am also appreciative of the National Watermelon Promotion Board and Appalachian State University Office of Student Research for providing funding for the present study.

Table of Contents

List of Figures

Chapter 1: Introduction

Menopause is considered the permanent end of menstruation; however, aging and the decrease in estrogen levels associated with menopause may also result in altered metabolism and adiposity. Menopause is also associated with an increase in the prevalence of obesity. Post-menopausal women over 60 years of age have an obesity rate of 73%, as compared to 65% for women between 40 and 59 years of age (Lizcano & Guzman, 2014). Estrogen has multiple effects in the body, including regulation of food intake, meal size, body weight, and distribution of fat deposits, in addition to the regulation of secondary sex characteristics and fertility (Butera, 2010).

Peptides associated with hunger and satiety have been reported to change with age and adiposity. Peptide YY (PYY) increases with age (Cahill et al., 2014), while, ghrelin and leptin decrease with age (Chedraui et al., 2014; Klok, Jakobsdottir, & Drent, 2007; Ostlund, Yang, Klein, & Gingerich, 1996). However, obese individuals have been reported to have decreased basal levels of PYY and increased leptin levels when compared to lean individuals (Batterham et al., 2003; Deibert et al., 2007). While not all of these changes would theoretically result in increased food intake, regulation of food intake is often complicated by changes in sensitivity to these peptides and their receptor content. More research is required to understand the effect of hunger and satiety peptides in obese, post-menopausal women.

In addition to increased overall obesity, post-menopausal women have decreased subcutaneous fat and increased abdominal fat compared to women of reproductive age (Lizcano & Guzman, 2014). Increased central adiposity is correlated with an increased risk of insulin resistance, metabolic syndrome, and type 2 diabetes (Al-Safi & Polotsky, 2014; Lizcano & Guzman, 2014; Toth, Poehlman, Matthews, Tchernof, & MacCoss, 2001). Post-menopausal women have an increased incidence of insulin resistance and metabolic syndrome compared to their premenopausal counterparts (Al-Safi & Polotsky, 2014; Sivasinprasasn et al., 2015; Twito, Frankel, & Nabriski, 2015).

Both obesity and insulin resistance are associated with chronic low-grade inflammation and oxidative stress (Paneni, Costantino, & Cosentino, 2015; Sivasinprasasn et al., 2015). In this state, oxidation of tetrahydrobiopterin (BH_4), a cofactor in the nitric oxide (NO) pathway, results in decreased of NO production (Katusic, 2001; Sansbury & Hill, 2014). NO can affect the regulation of food intake and insulin resistance (Abdul Razak & Saad; Cersosimo & DeFronzo, 2006; Jobgen et al., 2009; Morley, Alshaher, Farr, Flood, & Kumar, 1999; Sansbury & Hill, 2014; Wu et al., 2007). However, the effect of NO is dependent on how and where it is produced.

There are three nitric oxide synthase (NOS) isoforms that produce NO in the body. Endothelial NOS, eNOS, produces NO mostly in vascular endothelium for vasodilation; neuronal NOS, nNOS, is expressed mostly in skeletal muscle and neurons; and inducible NOS, iNOS, which has the highest capacity to produce NO, is expressed in many cell types

and is produced in response to inflammatory stimuli. Increased iNOS is associated with increased insulin resistance, increased glucose output from the liver and increased inflammation (Sansbury & Hill, 2014). In contrast, an increase in eNOS is associated with increased fat oxidation, suppressed gluconeogenesis, prevention of hyperinsulinemia, promotion of adipocyte browning, and increasing insulin and glucose delivery to skeletal muscle (Sansbury & Hill, 2014). In an obese individual, there is an increase in iNOS content and a down-regulation of eNOS (Sansbury & Hill, 2014). The changes in NOS expression and activity may be involved in inducing insulin resistance in obese individuals (Sansbury & Hill, 2014). NO has also been reported to increase food intake. Specifically, NO produced via nNOS decreases the effect of leptin in the hypothalamus (Calapai et al., 1998; Sansbury & Hill, 2014; Toutouzas, Riga, Stefanadi, & Stefanadis, 2008). While the exact role of NO in insulin and hunger and satiety signaling is not fully understood, it has emerged as a key metabolic regulator and potential intervention target.

Watermelon supplementation has been reported to stimulate the NO pathway and increase antioxidant capacity (Figueroa, Sanchez-Gonzalez, Perkins-Veazie, & Arjmandi, 2011; Figueroa, Sanchez-Gonzalez, Wong, & Arjmandi, 2012; Figueroa, Wong, Hooshmand, & Sanchez-Gonzalez, 2013; Figueroa, Wong, & Kalfon, 2014; Mohammad, Mohamed, & Zakaria, 2014). Watermelon contains large quantities of lycopene and beta-carotene (Mohammad, Mohamed, & Zakaria, 2014). These carotenoids are effective free radical scavengers (Mohammad, Mohamed, & Zakaria, 2014), which could aid in the production of NO. Free radicals can cause the uncoupling of tetrahydrobiopterin (BH4) and NOS, which

results in a decrease of the availability of NO (Holowatz, Thompson-Torgerson, & Kenney, 2010; Katusic, 2001; Mohammad, Mohamed, & Zakaria, 2014; Roe & Ren, 2012; Sansbury & Hill, 2014).

In addition, watermelon contains L-citrulline, which is the precursor of L-arginine in the NO pathway (Wu et al., 2007). L-arginine, along with BH4, oxygen, and nicotinamide adenine dinucleotide phosphate (NADPH), are necessary for NO production via the NOS (Katusic, 2001; Wijnands, Castermans, Hommen, Meesters, & Poeze, 2015). Supplementation of L-arginine has been reported to improve body composition by increasing muscle mass, decreasing fat mass, and improving insulin sensitivity in obese and diabetic rats (Sansbury & Hill, 2014). Therefore, I would expect watermelon, which is rich with L-arginine, to stimulate similar improvements in insulin sensitivity.

Watermelon also contains a significant amount of fructose. Fructose uptake by the liver is non-insulin dependent and does not cause a significant increase in insulin (Lustig, 2010). For this reason, fructose rich foods are typically recommended as an alternative to other sugar-containing foods for populations with insulin resistance (Le & Tappy, 2006); however, consuming large quantities of fructose could also have negative effects on post-menopausal health (Lustig, 2010). Insulin is part of the post-prandial satiety signaling (Sobrino Crespo, Perianes Cachero, Puebla Jimenez, Barrios, & Arilla Ferreiro, 2014). Fructose containing foods do not stimulate insulin secretion, resulting in decreased satiety and, therefore, increased food consumption (Lizarbe et al., 2013). This could have a negative

effect on body composition, and, therefore, insulin resistance (Le & Tappy, 2006; Lustig, 2010). In addition, fructose consumption drives de novo lipogenesis and the production of free fatty acids, which could also have negative effects on body composition and insulin resistance (Lustig, 2010). Watermelon consumption has been reported to improve oxidative stress and inflammation (Mohammad, Mohamed, & Zakaria, 2014), which may help with NO bioavailability. Watermelon could have a positive effect on insulin resistance by eNOS produced NO (Sansbury & Hill, 2014). However, if NO is produced through iNOS, insulin resistance may be exacerbated (Sansbury & Hill, 2014). Fructose consumption through watermelon could lead to decreased hepatic insulin sensitivity, increased free fatty acid production, and an overall decrease in insulin post-prandially (Stanhope et al., 2009). Therefore, fructose may have negative effects on hunger and satiety signals and body composition in post-menopausal, overweight women (Lustig, 2010).

Watermelon could have either a positive or negative impact on insulin resistance, depending on whether eNOS or iNOS stimulated NO production in post-menopausal, overweight women. However, based on previous research with diabetic rats (Wu et al., 2007), I hypothesize that watermelon consumption will improve insulin resistance in overweight, post-menopausal women. I also hypothesize fructose consumption through watermelon, would result in negative impacts on hunger and satiety signaling. Additionally, I hypothesize L-arginine will increase after six-weeks of watermelon supplementation, with no change in L-citrulline.

The purpose of this project was to investigate the metabolic effects of six weeks of watermelon supplementation. Our primary objective of this project was to investigate the effects of six weeks of watermelon supplementation on insulin resistance. Our secondary objective was to investigate the effects of watermelon supplementation on hunger and satiety signaling.

Chapter 2: Literature Review

Watermelon

Watermelon is a fruit that is rich in L-citrulline and L-arginine and has been investigated as a potential treatment of hypertension, oxidative damage, and metabolic syndrome (Collins et al., 2007; Figueroa et al., 2011; Figueroa et al., 2012; Figueroa et al., 2013; Figueroa et al., 2014; Mohammad, Mohamed, Zakaria, Abdul Razak, & Saad, 2014; Wu et al., 2007). L-citrulline is a precursor of L-arginine, which is utilized in the production of NO (Wu & Morris, 1998). After ingestion, approximately 38% of L-arginine is catabolized by intestinal and liver tissue before entering the systemic circulation (Castillo et al., 1993). L-citrulline catabolism is limited; thus, more enters portal circulation in adults (Collins et al., 2007). Due to the limited degradation of L-citrulline, it is available for conversion to L-arginine, which can then be used to produce NO (Collins et al., 2007). NO has been reported to improve insulin sensitivity and increase mitochondrial biogenesis when produced via eNOS (Jobgen et al., 2009; Sansbury & Hill, 2014); however, an increase in iNOS could have a negative effect on insulin sensitivity, and an increase in nNOS could negatively affect satiety signaling (Sansbury & Hill, 2014).

Watermelon also contains the free radical scavengers lycopene and beta-carotene (Perkins-Veazie, Collins, Pair, & Roberts, 2001). Free radical scavengers are utilized in an antioxidant defense mechanism that maintains redox balance and may decrease free radical toxicity (Mohammad, Mohamed, Zakaria, et al., 2014). Reactive oxygen species (ROS) are oxygen-containing molecules that have oxidative effects (Oyinloye, Adenowo, & Kappo,

2015; Powers, Nelson, & Hudson, 2011). Free radical scavengers decrease ROS by donating an electron from one of multiple double bonds, which stabilizes the ROS. When ROS exceed the cellular antioxidant capacity, oxidative stress occurs, which can lead to inflammatory diseases (Oyinloye et al., 2015). Mohammad et al. showed that watermelon supplementation can decrease oxidative stress (Mohammad, Mohamed, Zakaria, et al., 2014).

Watermelon also contains a variety of sugars, specifically sucrose, glucose, fructose and maltose. Humans metabolize these carbohydrates by different mechanisms. Glucose is utilized throughout the body, especially in the brain, skeletal muscle, and liver. Fructose is only metabolized by the liver (Lustig, 2010). Fructose is brought into the liver by non-insulin dependent glucose transporters (Glut5) and is converted to fructose-1-phosphate through hydrolysis of adenosine triphosphate (ATP) (Lustig, 2010). As ATP decreases, adenosine monophosphate (AMP) increases and is then metabolized to uric acid and then urate (Taylor & Curhan, 2008); urate buildup in circulation inhibits eNOS (Lustig, 2010). Decreased eNOS can lead to insulin resistance (Sansbury & Hill, 2014). Because fructose transport is non-insulin dependent, fructose-containing food do not stimulate insulin secretion (Stanhope et al., 2009). Insulin is part of the satiety signal (Lizarbe et al., 2013). Therefore, decreased insulin could result in decreased satiety and thus, increased food consumption. In addition, high fructose ingestion results in to lower leptin levels compared to high glucose post-ingestion (Moran, 2009). This could have a negative effect on body composition, and therefore, on insulin resistance (Le & Tappy, 2006; Lustig, 2010). Excess fructose is the primary driver of de novo lipogenesis in the liver (Lustig, 2010). Fructose

stimulates peroxisomal proliferator-activated receptor-γ coactivator-1β, a transcriptional coactivator of SREBP-1, which stimulates de novo lipogenesis (Nagai et al., 2009); thus, excess watermelon could negatively impact insulin sensitivity with increased circulating triglycerides that are produced due to increased fructose consumption.

L-arginine and NO

L-arginine is an amino acid utilized in the production of NO throughout the body (Boger, 2014; Katusic, 2001). NO is produced through nitric oxide synthase (NOS) enzymes of which there are three isozymes: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). Briefly, NOS combines L-arginine, BH4, oxygen and NADPH to form NO and L-citrulline (Katusic, 2001; Wijnands et al., 2015). The biological effect of NO is dependent on how and where it is produced and will be discussed in relation to insulin resistance and intake signaling in detail below.

About 60% of L-arginine is produced in the kidneys; however, almost every cell is capable of producing L-arginine by recycling L-citrulline via arginosuccinate synthase and arginosuccinate lyase (Wu & Morris, 1998). Briefly, L-citrulline is converted to L-argininosuccinate via arginiosuccinate synthase (ASS), which is then converted to L-arginine via argininosuccinate lyase (ASL) (Wu & Morris, 1998). Despite the body's ability to produce L-arginine, L-arginine supplementation has been reported to increase the production of NO. This increase in L-arginine can be utilized by each NOS isoform; however, L-

arginine supplementation has been reported to increase eNOS expression in rats, but additional human studies are needed (Boger, 2014).

Post-menopausal Women

Menopause is noted by the decrease of estrogen and the cessation of a menstruation cycle for 12 months (MayoClinic, 2015). Menopause can occur around the age of 50 with the average age being 51 in the United States (MayoClinic, 2015). In addition to reproductive changes, post-menopausal women are three times more likely to develop obesity and metabolic syndrome when compared to premenopausal women (Eshtiaghi, Esteghamati, & Nakhjavani, 2010; Lizcano & Guzman, 2014).

Estrogen plays a role in distribution of fat, with post-menopausal women showing an increased level of abdominal fat (Cervellati et al., 2009; Ley, Lees, & Stevenson, 1992) and a decreased levels of subcutaneous fat (Lizcano & Guzman, 2014). Svendsen et al. reported no change in abdominal fat or total body fat in healthy premenopausal women but suggested the increase in total body and abdominal fat occurred during perimenopause and may continue to increase after the onset of menopause (Svendsen, Hassager, & Christiansen, 1995). Increased abdominal adiposity is associated with increased insulin resistance, metabolic syndrome, and type 2 diabetes (Lizcano & Guzman, 2014; Paneni et al., 2015; Sansbury & Hill, 2014; Sivasinprasasn et al., 2015). Post-menopausal women have a higher prevalence of both insulin resistance and diabetes (Kim & Feldman, 2015; Sivasinprasasn et al., 2015; Twito et al., 2015). In post-menopausal women, increased adiposity, free radicals,

and age may all contribute to the prevalence of increased insulin resistance, diabetes, and metabolic syndrome (Kim & Feldman, 2015; Sivasinprasasn et al., 2015; Twito et al., 2015).

Changes in estrogen influence food intake with increased estrogen in proestrus leading to decreased food intake, while, ovariectomized rats experience increases in food intake and body weight gain (Butera, 2010). Food intake and satiety signaling peptides can be affected by both age and adipose level (Cahill et al., 2014; Chedraui et al., 2014; Deibert et al., 2007; Klok et al., 2007). Cahill et al. reported increases in satiety signaling PYY with age, and PYY is positively correlated with body fat, trunk fat, and waist circumference (Cahill et al., 2014). This contradicts Batterham et al., who reported a negative correlation with PYY and BMI (Batterham et al., 2003). Cahill et al. noted that post-menopausal women have higher PYY concentrations than premenopausal women, which may be the result of a combination of confounding variables such as medication, age, and smoking status (Cahill et al., 2014). Ostlund et al. reported that with increasing age, the satiety peptide leptin levels decrease, but leptin increases with increased adiposity (Ostlund et al., 1996). Deibert et al. compared premenopausal and post-menopausal women trying to lose weight. The post-menopausal women had more leptin compared to the premenopausal women (Deibert et al., 2007). When comparing pre-menopausal and post-menopausal women, body weights in both groups were similar; however, the post-menopausal group had more body fat (Deibert et al., 2007). These results would suggest leptin is more influenced by body composition than by age (Deibert et al., 2007; Klok et al., 2007). Ghrelin, a hunger stimulating peptide, has been

reported to decrease with age (Klok et al., 2007). If leptin increases while ghrelin decreases in post-menopausal women, this would decrease appetite, leading to less food consumption.

Insulin Resistance

Insulin resistance (IR) is a condition in which normal levels of insulin do not illicit the normal biological response (Al-Jiffri, Al-Sharif, Abd El-Kader, & Ashmawy, 2013). Insulin, an anabolic hormone, is essential for glucose homeostasis, growth, and regulation of fat and protein metabolism (Saltiel & Kahn, 2001). Insulin is secreted from the pancreas in response to a rise in blood glucose and stimulates an increase in glucose uptake in muscle and adipose tissue (Saltiel & Kahn, 2001). Low levels of insulin resistance can persist for years with blood glucose at or near normal levels (Nichols, Hillier, & Brown, 2008). Over time and with increased severity, insulin resistance may eventually progress to type 2 diabetes (Shaw, Zimmet, McCarty, & de Courten, 2000). Obesity and insulin resistance are associated with increased oxidative stress and inflammation. This can lead to decreased NO availability. In a state of oxidative stress, the NO cofactor BH4 can be oxidized into BH2 via superoxide, resulting in uncoupling of the NOS dimer (Roe & Ren, 2012). NO can also be oxidized by superoxide anion to produce perioxynitrite anion; thus, increased free radicals can decrease the availability of NO to its target tissues (Katusic, 2001). Insulin resistance is commonly measured using the homeostatic model assessment of insulin resistance (HOMA-IR). Various models that test insulin resistance include euglycaemic clamp, Minimal Model (MinMod), continuous infusion of glucose with model assessment (CIGMA), and HOMA (Hermans, Levy, Morris, & Turner, 1999). HOMA is a relatively inexpensive, simple model

that utilizes fasting blood glucose and insulin concentrations to estimate β-cell function (HOMA-%β) as well as insulin resistance (HOMA-IR) by using a mathematical formula (Hermans et al., 1999; Matthews et al., 1985; Song et al., 2007; Wallace, Levy, & Matthews, 2004).

NO and insulin resistance

As previously described, the biological effect of NO is NOS isozyme dependent. Overweight individuals have a decrease in eNOS expression due to an increase in tumor necrosis factor-α (TNFα), which decreases eNOS mRNA stability (Yoshizumi, Perrella, Burnett, & Lee, 1993). Deletion of eNOS in mice has been reported to cause insulin resistance (Duplain et al., 2001).

It has been suggested that eNOS has several influences on insulin sensitivity (Sansbury & Hill, 2014). Insulin activates eNOS, which produces NO dependent vasodilation, aiding in insulin delivery and utilization in various tissues (Wang, Wang, Aylor, & Barrett, 2013). Intra- and extracellular eNOS produced NO, which inhibits protein-tyrosine phosphate 1b (PTP1B), an inhibitor of intracellular insulin signaling, although the exact mechanism is unclear (Wang et al., 2013).

Cells that produce an inflammatory response, like immune cells, have iNOS present and is upregulated in obesity (Sansbury & Hill, 2014). Pro-inflammatory cytokines have been shown to increase iNOS and has been hypothesized to induce insulin resistance (Perreault & Marette, 2001). Ropelle et al. have reported increased iNOS expression leads to

increased skeletal muscle insulin resistance (Ropelle et al., 2013). Overexpression of iNOS in the liver has been reported to cause insulin resistance in mouse liver (Shinozaki et al., 2011). There is evidence that insulin resistance in type 2 diabetes is linked with iNOS dependent S-nitrosylation of early signaling molecules such as PKB/Akt, the insulin receptor β-subunit (IRβ), and insulin substrate receptor-1 (IRS-1) (Carvalho-Filho, Ueno, Carvalheira, Velloso, & Saad, 2006; Carvalho-Filho et al., 2005). These key insulin signaling pathway regulators are disrupted with the increase in NO, which could ultimately become a contributing factor of insulin resistance (Carvalho-Filho et al., 2005; Ropelle et al., 2013).

L-arginine supplementation has been reported to enhance NO production, but it is catabolized early in intestinal metabolism (Katusic, 2001; Wijnands et al., 2015; Wu & Morris, 1998). L-citrulline is not metabolized as early and can be converted to arginine for NO production (Castillo et al., 1993; Wu & Morris, 1998). If NO is produced through eNOS, insulin sensitivity may improve; however, if NO production through iNOS is enhanced, then insulin resistance may worsen (Sansbury & Hill, 2014). Therefore, when supplementing with watermelon, the NOS isozyme utilized will dictate the effect on insulin resistance.

Hunger and satiety signaling

The hypothalamus is the primary regulator of food and utilizes several hormones to control food selection and intake, ultimately effecting body weight (Adamska, Ostrowska, Gorska, & Kretowski, 2014). Hormones produced in adipose tissue and the gastrointestinal

system are known to influence neural signals of hunger and satiety (Ezcurra, Reimann, Gribble, & Emery, 2013; Thomas & Schauer, 2010; Tolhurst et al., 2011; Troke, Tan, & Bloom, 2014). From the gastrointestinal system, ghrelin stimulates increased food intake, while GLP-1 and peptide YY increase satiety (Ezcurra et al., 2013; Tolhurst et al., 2011; Troke et al., 2014). Leptin, produced via adipocytes, also increases satiety, reducing food intake (Lizarbe et al., 2013). GIP is produced in the gastrointestinal system, however, by itself does not have an effect on hunger or satiety directly, but does stimulate the secretion of insulin, leading to a potential satiety effect (Chiang, Ip, & Jin, 2012; Lizarbe et al., 2013).

Ghrelin

Ghrelin is a hormone that is secreted mainly by the stomach, which stimulates appetite, and is highly dependent on the nutritional state (Klok et al., 2007). Ghrelin has a diurnal component and is decreased with increasing age, BMI, glucose, and insulin levels (Klok et al., 2007; Soni, Conroy, Mackey, & Kuller, 2011). Women have higher levels of ghrelin compared to men (Klok et al., 2007).

Ghrelin levels have been reported to be lower in obese individuals compared to their leaner counterparts (Tschop et al., 2001), and other studies have reported increased ghrelin levels with weight loss (Klok et al., 2007). Ghrelin increases with discontinuation of hormone replacement therapy in post-menopausal women with metabolic syndrome (Chedraui et al., 2014; Soni et al., 2011). This suggests that menopause may be a strong regulator of ghrelin secretion as compared to obesity in post-menopausal women.

GIP (total)

Gastric inhibitory peptide (GIP) was described by its initial characteristic of inhibition of histamine-induced gastric acid (Gault, O'Harte, & Flatt, 2003). GIP is considered an incretin hormone due to its augmentation of postprandial insulin secretion (Irwin & Flatt, 2013); thus, the name was changed to a more suitable alternative, "glucose-dependent insulinotropic polypeptide" (Pederson & Brown, 1976). GIP is secreted from the enteroendocrine K-cells of the duodenum in response to food intake (Rudovich, Kaiser, Engeli, Osterhoff, Gögebakan, et al., 2007) and is regulated largely by carbohydrates and fat (Irwin & Flatt, 2009). GIP is triggered to be released by Na^+-coupled glucose uptake mediated by the brush border sodium glucose cotransporter (SBLT1) (Ezcurra et al., 2013). GIP concentrations vary, depending on the size and composition of the meal and health status (Vilsboll, Krarup, Deacon, Madsbad, & Holst, 2001). After entering circulation, GIP is degraded rapidly by the ubiquitous enzyme dipeptidyl peptidase 4 (DPP-4), producing GIP(3-42), which does not have the insulinotropic effect (Irwin & Flatt, 2013). GIP and its metabolites are also cleared by the body via renal filtration (Irwin & Flatt, 2013). GIP has been reported to stimulate proinsulin gene transcription and translation, beta cell growth, differentiation, proliferation and survival (Irwin & Flatt, 2009).

GIP receptors are prevalent in the stomach, pancreas, and adipocytes (Irwin & Flatt, 2009). There is growing evidence to support GIP receptor-mediated effects that link consumption of high-fat diets and the development of obesity, insulin resistance and type 2 diabetes (Irwin & Flatt, 2009). Rudovich et al. have reported different gene expression in

various adipose tissues with decreased GIP receptor gene expression in subcutaneous fat and higher levels of GIP receptor genes in visceral fat (Rudovich, Kaiser, Engeli, Osterhoff, Gögebakan, et al., 2007). The increased GIP receptor gene expression in visceral fat was positively correlated with fasting insulin, glucose, LDL concentrations and HOMA-IR (Rudovich, Kaiser, Engeli, Osterhoff, Gögebakan, et al., 2007); however, the decreased expression of GIP receptors in subcutaneous fat is associated with insulin resistance (Rudovich, Kaiser, Engeli, Osterhoff, Gögebakan, et al., 2007).

GLP-1 (active)

Glucagon-like peptide-1 (GLP-1) is an incretin hormone with an ability to increase insulin secretion (Ezcurra et al., 2013). GLP-1 is a hormone that is broken down from the prohormone proglucagon (Lee & Jun, 2014). The GLP-1 hormone has two active forms, GLP-1 (7-37) and the major active form GLP-1 (7-36) (Lee & Jun, 2014). The gut and brain process proglucagon to produce GLP-1 (Lee & Jun, 2014). GLP-1 is released from L-cells in the small intestine and colon and α-cells in the pancreas and in the brain in response to food ingestion (Ezcurra et al., 2013; Lee & Jun, 2014). GLP-1 is also secreted in response to both fats and protein, specifically L-glutamine; however, the amount of protein needed to optimize GLP-1 release is unclear (Ezcurra et al., 2013; Tolhurst et al., 2011). GLP-1 is released in a biphasic pattern, with the first phase occurring 10-15 minutes after oral food ingestion and the second occurring 30-60 minutes after oral food ingestion (Lee & Jun, 2014). The initial secretion of GLP-1 may be due to vagus nerve innervation rather than direct stimulation of

L-cells (Rocca & Brubaker, 1999). Fasting concentrations of bioactive GLP-1 can increase as much as 3-fold, depending on the last meal consumed (Elliott et al., 1993).

GLP-1 has many effects on appetite and metabolism in the body, including delaying gastric emptying, inhibiting food intake, improving insulin sensitivity, stimulating insulin synthesis, and inhibiting glucagon secretion (Lee & Jun, 2014). GLP-1 receptors are located throughout the body and include the central and peripheral nervous system, the GI tract, muscle, adipose tissue, pancreas, liver, bone, and the cardiovascular system (. Kim & Egan, 2008). In the central nervous system, GLP-1 decreases appetite and stimulates neural information to be conveyed to other target tissues, like the pancreas (. Kim & Egan, 2008; Zander, Madsbad, Madsen, & Holst, 2002). In the GI tract, GLP-1 has been reported to inhibit gastric acid secretion, gastric emptying and motility (. Kim & Egan, 2008; Wettergren et al., 1993). In the pancreas, GLP-1 has a central role in β-cell mass with increased proliferation with decreased β-cell apoptosis (. Kim & Egan, 2008).

GLP-1 is eliminated via hepatic and renal clearance and is cleaved via DPP-4, resulting in the short half-life of approximately 2 minutes (Ezcurra et al., 2013; Lee & Jun, 2014). GLP-1, unlike other gut hormones, does not seem to be affected by excess body fat (Troke et al., 2014).

Leptin

Leptin is produced from white adipocytes and is released in proportion to the amount of body fat (Dalamaga et al., 2013; Park & Ahima, 2014). Leptin has many signaling

pathways that affect, the brain, specifically the hypothalamus, and peripheral tissues like adipocytes and skeletal muscle (El-Haschimi, Pierroz, Hileman, Bjorbaek, & Flier, 2000; Moon et al., 2013; Park & Ahima, 2014). Women have higher levels of leptin than men due to increased subcutaneous adipose tissue, which secretes leptin (Deibert et al., 2007; Leshan, Bjornholm, Munzberg, & Myers, 2006; Moon et al., 2013; Ostlund et al., 1996). Post-menopausal women have also been reported to have increased leptin compared to premenopausal women (Deibert et al., 2007). Age, sex, and caloric restriction may alter leptin levels, but these are secondary regulators compared to increased adiposity (Ostlund et al., 1996). Leptin levels are also increased via increases in insulin and pro-inflammatory cytokines (TNF-α and interleukin-1) (Ahima & Osei, 2004; Paz-Filho et al., 2012).

Leptin functions as a feedback mechanism that signals the brain to inhibit food intake and regulate body weight and energy homeostasis (Klok et al., 2007). Leptin deficient mice and humans are severely obese and have endocrine alterations such as hyperglycemia and insulin resistance (Paz-Filho et al., 2012). Obese individuals have significantly higher circulating leptin than their lean counterparts; however, the increased leptin does not suppress feeding or weight gain (Ahima & Osei, 2004) due to leptin resistance (Sainz, Barrenetxe, Moreno-Aliaga, & Martinez, 2015).

Leptin resistance is described as the diminished effect of leptin's ability to suppress appetite and weight gain as well as to stimulate energy expenditure (Moon et al., 2013). The mechanism for leptin resistance remains unclear, but several theories have been postulated,

which include impaired ability to cross the blood brain barrier, an inhibition of leptin signaling in the brain and decreased cellular downstream signaling response (El-Haschimi et al., 2000; Sainz et al., 2015). Leptin resistance can lead to hyperphagia, impaired nutrient absorption, and impaired glucose and lipid metabolism (Moon et al., 2013; Sainz et al., 2015). Leptin resistance has been reported in rats fed a high-fructose diet (Sainz et al., 2015).

Leptin and insulin have been suggested to have overlapping signaling pathways, and leptin has been reported to improve glucose tolerance when administered as a therapy in mice (Keung, Palaniyappan, & Lopaschuk, 2011). Leptin administration has been reported, in vivo, to stimulate insulin's inhibitory effect on hepatic glucose output while inhibiting insulin's effect on glucokinase (Moon et al., 2013). Leptin treatment has been reported to induce phosphorylation of Akt and glycogen synthase kinase 3 but not to the same degree as insulin (Szanto & Kahn, 2000). In the liver, leptin antagonizes insulin, while in the muscle, leptin stimulates a non-IRS-1-associated PI3K, which mimics glucose transport (Moon et al., 2013). This suggests a complex signaling interaction between leptin and insulin that is tissue specific, with some interactions being proposed to be involved in the development of insulin resistance (Moon et al., 2013).

PYY (total)

Peptide YY (PYY) is a hormone that is secreted by the L cells in the distal small intestine (Troke et al., 2014). While all macronutrients increase secretion of PYY, the

stimulus for the most potent release in obese humans is a high carbohydrate, low fat diet (Essah, Levy, Sistrun, Kelly, & Nestler, 2007). PYY levels begin to rise within 15 minutes of food ingestion, which implies a neural or hormonal mechanism for its release (Gibbons et al., 2013). PYY works to decrease gastric emptying, delaying the transition from the stomach to the intestine and to decrease ghrelin levels (Troke et al., 2014). PYY levels peak around 60 minutes after food ingestion and remain elevated for up to six hours (Adrian et al., 1985).

Fasting levels of PYY are lower in obese individuals compared to lean individuals, and there is also a blunted response in obese individuals (Batterham et al., 2003; Hill, De Souza, Wagstaff, Sato, & Williams, 2012; Troke et al., 2014). Infusion of PYY has been reported to inhibit appetite and food intake in both lean and obese individuals (Batterham et al., 2003). It is uncertain whether low PYY leads to obesity or obesity leads to low PYY (Batterham et al., 2003).

NO and Hunger/satiety signaling

NO does not increase or decrease hunger or satiety peptides production or secretion. In the periphery GIP and GLP-1 are not dependent on NO for secretion, affecting gastric emptying, or the incretin response of elevating insulin (Gentilcore et al., 2005). However, the central nervous system utilizes NO for some of the processes involved in regulation of feeding (Morley, Farr, Sell, Hileman, & Banks, 2011). Specifically, NO affects the function of leptin and ghrelin (Farr, Banks, Kumar, & Morley, 2005; Morley et al., 2011). In the

hypothalamus, ghrelin has been reported to increase NOS levels thus increasing NO, while leptin decreases NOS thus decreasing NO (Gaskin, Farr, Banks, Kumar, & Morley, 2003; Morley et al., 2011). NO does not directly affect the activity of these signaling molecules but could be a limiting factor if the substrates required for the production of NO were deficient. Therefore, if watermelon supplementation changes the availability of L-arginine in the brain, food intake signaling in the CNS could be altered. Also, as previously mentioned, fructose can have a negative impact on inflammation and oxidative stress, which can lead to the uncoupling of BH_4 and NOS, resulting in a decrease in NO. Fructose also can exacerbate insulin resistance in the liver, in skeletal muscle and decrease post-prandial insulin secretion (Stanhope et al., 2009).

Lastly, both insulin and leptin utilize eNOS in their signaling pathways, but in opposition to the other (Mehebik-Mojaat, Ribiere, Niang, Forest, & Jaubert, 2009). Leptin, when stimulating eNOS, inhibits IRS-1 via PKA, which inhibits insulin signaling (Mehebik-Mojaat et al., 2009). When insulin stimulates eNOS, JAK2-P is inhibited via SHP-1 through the insulin receptor thus inhibiting leptin signaling (Mehebik-Mojaat et al., 2009). This interference effect could be the link between obesity and insulin- and leptin-resistance (Joffin, Niang, Forest, & Jaubert, 2012). Jobgen et al. have reported decreased leptin levels and improved glucose disposal in male rats with L-arginine supplementation (Jobgen et al., 2009). Jobgen et al. suggested improved glucose disposal was credited to improved insulin sensitivity and increase in muscle mass (Jobgen et al., 2009).

NO should have no effect on hunger and satiety signaling peptides, except insulin. The upregulation of insulin, however, could effect other hunger and satiety hormone concentrations and pathways. Fructose may also impact the hunger and satiety signals, especially insulin. Thus, fructose may negatively impact the hunger and satiety peptides.

Conclusion

Women experience many changes with the onset of menopause, including changes in food intake and body composition. With the increased prevalence in obesity and central adiposity in post-menopausal women, there is an increased risk of insulin resistance, metabolic syndrome, and type 2 diabetes (Lizcano & Guzman, 2014). L-arginine has been reported to improve insulin resistance and metabolic syndrome through NO pathways (Sansbury & Hill, 2014; Wu et al., 2007). Watermelon is high in L-citrulline, a precursor to L-arginine, which is a substrate for the NO pathway (Figueroa et al., 2011; Figueroa et al., 2012; Figueroa et al., 2013; Figueroa et al., 2014; Wu et al., 2007); however, the effects of watermelon supplementation on insulin resistance and hunger satiety signaling are unknown. We predict there will be an improvement in insulin resistance with the production of NO through eNOS with watermelon supplementation. We also predict hunger and satiety peptides will be negatively impacted due to fructose present in watermelon. Additionally, we hypothesize L-arginine will increase after six-weeks of watermelon supplementation, with no change in L-citrulline. Therefore, the purpose of this study is to investigate the effects of watermelon supplementation in post-menopausal, overweight women.

Chapter 3: Methods

Subjects

Sixty overweight (BMI >25), post-menopausal women, with ages of 50-75 were recruited. After completing a voluntary informed consent form, subjects were required to complete a medical health questionnaire. A medical health questionnaire was used to verify medical history and lifestyles of each subject and was compared to the exclusion criteria. Subjects were non-smokers with no overt diagnosed chronic medical conditions such as diabetes, cardiovascular disease, hypertension, cancer, or rheumatoid arthritis. Subjects were excluded if they regularly consumed large quantities of L-citrulline/L-arginine rich foods or supplements. Subjects were excluded if they took exogenous ovarian hormones, antihypertension drugs, or medications known to influence inflammation. Subjects were also excluded if they were allergic to watermelon, attempting to lose body weight, or consumed weight-loss medications. This study was approved by Appalachian State University's Institutional Review Board (14-0092).

Experimental Design

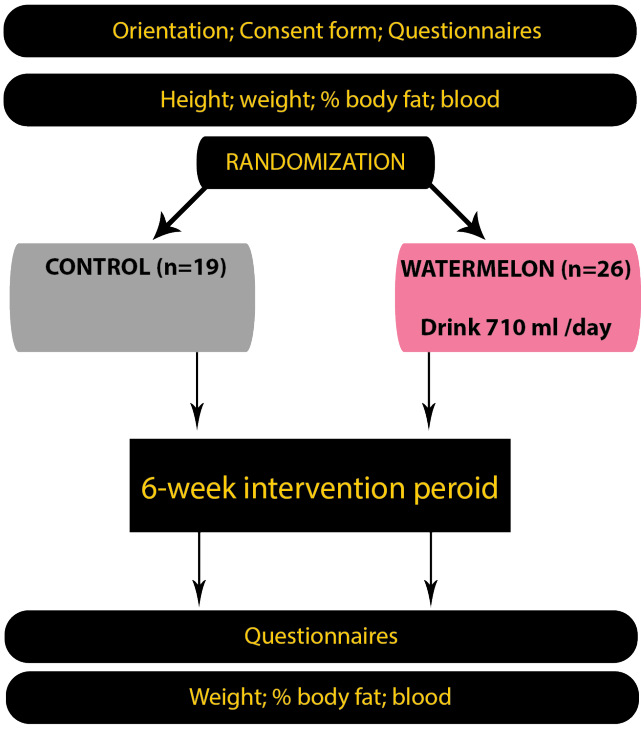

Figure 1. Schematic of study design. Pre-intervention visit consisted of informed consent, orientation and questionnaires, collection of anthropometric data and a fasting blood draw. Subjects were randomized assigned into groups and provided watermelon (WM), if in the WM group. Following six-week intervention subjects returned for their post study assessments.

Upon arrival to lab for the pre-intervention visit, the subjects were asked to complete informed consent forms, orientation and questionnaires. The subject's anthropometric data were collected, including height and weight and body fat percentage (Tanita Corporation, Tokyo, Japan, TanitaTBF-300A). The subjects were randomly assigned into either the watermelon group or the control group. A trained phlebotomist drew blood. Blood was collected in serum separating tubes and EDTA tubes and processed accordingly. The

watermelon group was given a six-week supply of watermelon puree. Both groups were asked to limit L-arginine rich foods, such as red meat, peanuts, walnuts and tuna. A dose of 1.88g of L-citrulline and 0.39g of L-arginine (710ml of watermelon puree) was consumed each day.

Watermelon supplement

Millionaire seedless sunripe watermelons (Falls Church, VA, USA) were utilized for a watermelon puree and provided via a pilot plant at the U.S. Department of Agriculture Citrus and Subtropical Products Laboratory (Winterhaven, FL, USA). Watermelon was cold pressed pasteurized and analyzed for L-citrulline and L-arginine concentrations (Dr. Penelope Perkins-Veazie- Plants for Human Health Institute, North Carolina State University). Watermelon puree was stored at -20^{o}C before distribution to the subjects. Subjects were instructed to keep watermelon puree frozen until ready for consumption, at which time the puree should be thawed with running hot water over the bottle. Each bottle of watermelon puree, 710mL, contains 2.27g of combined L-citrulline/L-arginine, specifically, 1.88g of L-citrulline and 0.39g of L-arginine.

Blood collection

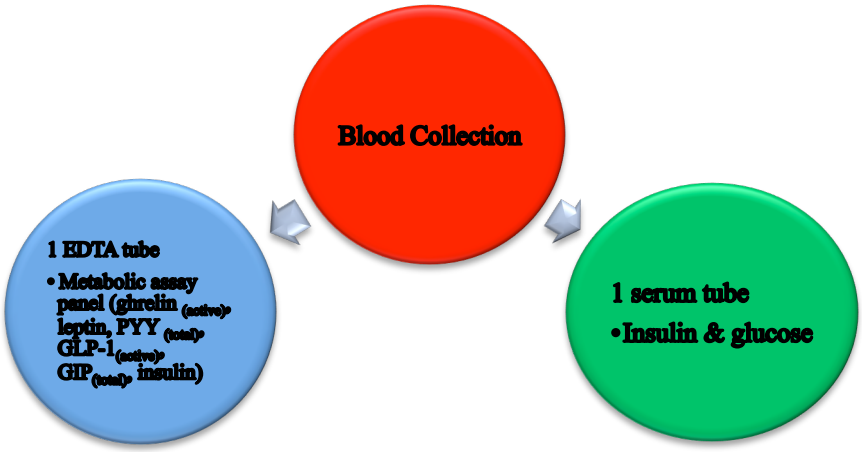

Figure 2. Schematic of blood collection and the types of tubes utilized for various samples.

Blood samples were separated into the collection tubes listed above. Protease inhibitors (serine protease inhibitor and DPPIV inhibitor) were added to the EDTA blood collection tubes. Samples were mixed by inversion and then separated by centrifugation. The EDTA tubes were centrifuged at 1000 relative centrifuge force (rcf) for ten minutes at 4^{o}C. Plasma from the EDTA tubes was aliquoted, flash frozen in liquid nitrogen, and stored at -80^{o}C. The serum separator tubes were centrifuged at 1000rcf at room temperature. These tubes were transported at room temperature to Cannon Memorial Hospital and were analyzed for fasting blood glucose and insulin levels.

Metabolic assay

Plasma aliquots for the metabolic panel assay were analyzed using a Milliplex human metabolic hormone magnet bead panel to test for active ghrelin, GIP, active GLP-1, insulin, leptin and total PYY (MILLIPLEX® Map Kit – Human Metabolic Hormone Magnetic Bead Panel; EMD Millipore, Billerica, MA). Briefly, wells were washed with assay buffer. A 25µL volume of standards and controls were placed into the appropriate wells. A 25µL volume of assay buffer was added to the sample wells, along with 25µL of the plasma samples. A 25µL volume of matrix solution was added to the standards and controls. The magnetic beads, one for each analyte of interest, were mixed. The beads were pre-conjugated with the necessary primary antibodies. A 25µL volume of the mixed magnetic beads were added to each well. The plates were then sealed and covered with aluminum foil to protect the beads from light. The plates were agitated on a plate shaker at 4^{o}C overnight, which allowed for analyte-bead binding. After the overnight incubation, the plates were washed three times to remove all excess, non-specific analytes in solution. The plate was placed on a strong handheld magnet for one minute to ensure the beads had settled to the bottom of the plate before decanting. The plate was decanted with the magnet still beneath the plate to ensure no beads were lost. Next, the plate was placed upright, removed from the magnet and 200µL of assay buffer was added to each well. The plate was agitated for 30 seconds on a plate shaker. After agitation, the plate was placed on the magnet for one minute. The assay buffer was decanted, and this process was repeated for a total of three washes. A 50µL volume of detection antibodies were added to each well. The plate was

sealed, covered with aluminum foil and agitated for one hour on the plate shaker at room temperature. This allowed for equilibrium to be reached between the binding and unbinding secondary antibodies to the primary antibody-analyte complex. After the incubation, 50µL of Streptavidin-Phycoerythrin, the detection molecule, was added to each well. Streptavidin-Phycoerythrin binds to the detection antibody. The plates were sealed, covered with aluminum foil and agitated for 30 minutes at room temperature. After the incubation, the plate was decanted and another wash cycle was performed. A 100µL volume of wash buffer was added to all wells. The plates were sealed, covered with aluminum foil and agitated on the plate shaker for 5 minutes to resuspend the beads. The samples were analyzed using the MAGPIX® instrument and xPONENT® analysis software (Luminex, Austin, TX). Briefly, a portion of the sample was removed from the well and ejected it into the analyzing chamber. A magnet was applied to form a monolayer of beads. A red light emitting diode, LED, was used to illuminate the chamber. The type of bead was determined based on color emitted in response to the red LED. Following the illumination from the red LED, a green LED illuminated the chamber. Based on the color the beads emitted from the green LED, the concentration of the analyte was determined.

HOMA-IR

Serum blood tubes were sent to Cannon Memorial Hospital Laboratory to obtain fasting blood glucose and insulin levels. Insulin was determined using an electrochemiluminescence immunoassay. During the first incubation, samples were added to

a mixture of biotinylated monoclonal insulin-specific antibodies and a monoclonal insulin-specific antibody labeled with ruthenium complex. The second incubation was after the addition of the detector molecule streptavidin, which then bound to the complex.

Fasting blood glucose was determined via an enzymatic reference method, utilizing hexokinase and UV testing to determine concentrations. Briefly, glucose is converted to glucose-6-phosphate when in the presence of ATP and hexokinase. Glucose-6-phosphate and $NADP^+$ is converted to gluconate-6-phosphate and $NADPH+H^+$ via glucose-6-phosphate dehydrogenase. The NAPDH is analyzed photometrically at 340nm. NAPDH is directly proportional to the glucose concentration.

These values were used to calculate homeostatic model assessment-estimated insulin resistance, HOMA-IR. HOMA-IR was calculated using the following formula: fasting insulin (μIU/ml) × fasting glucose (mg/dl)/405 (S. W. Lee, Jo, Kim, Kim, & You, 2015)

Statistics

A 2x2 (treatment x time), repeated measures ANOVA was performed using SPSS (IBM Corp. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY) to determine if differences existed between groups. If significance was found, the Fisher LSD post-hoc test was performed. Significance was set at $p \leq 0.05$. Effect size was determined only in results that showed a time-by-treatment interaction by Cohen's D (Cohen, 1988). Data are expressed as percent change ± SD, with the exception of subject characteristics, which are expressed as mean ± SD.

Chapter 4: Results

Subject characteristics

After analysis of blood glucose and insulin, six subjects with fasting blood glucose >125 were excluded because of the potential for undiagnosed diabetes (control, n=2, watermelon, n=4). There were no significant differences between treatment groups for age or BMI. However, there was a main effect of time to increase weight (% change=0.5±1.4%, p=0.048), and body fat percentage (% change=1.1±2.8%, p=0.007) at the end of the six-week period (Table 1).

Table 1. Subject Characteristics

	Pre-Study	Post-Study	Time; interaction effects, P-Value[a]
Age			
CT	60.1 ± 1.6		
WM	59.5 ± 1.0		
Mass (kg)			
CT	82.6 ± 3.3	82.7 ± 3.3	0.048; 0.297
WM	84.2 ± 3.4	84.8 ± 3.4	
BMI (kg/m^2)			
CT	30.3 ± 1.4	30.4 ± 1.1	0.285; 0.351
WM	29.9 ± 1.2	31.1 ± 0.92	
Body Fat %			
CT	42.6 ± 1.1	43.3 ± 1.2	0.007; 0.259
WM	42.9 ± 0.95	43.2 ± 1.0	

Notes: All data are means ± SE. [a]The first P-value represents the overall time effect; the second P-value represents the condition (control vs. watermelon) × time (2 time points) interaction effects. CT = Control, WM = Watermelon

Watermelon measurements

L-arginine significantly increased due to a time-by-treatment interaction in the watermelon group (% change=8.3 ± 11.4%, p=0.005) (Figure 3). The effect size was small (d=0.349). L-citrulline did not change (% change: CT=1.7 ± 16.2% vs. WM=1.8 ± 10.4%) (Figure 4).

Glucose, Insulin and Insulin resistance

Fasting blood glucose did not change (% change: CT =-0.6 ± 5.4% vs. WM=0.3 ± 6.8%) (Figure 5). There was a main effect of time to increase insulin levels (% change= 13.0 ± 30.1%, p=0.05) (Figure 6). HOMA-IR did not change significantly, but there was a trend toward a main effect of time (% change=25.2 ± 35.9%, p=0.071) (Figure 7).

Hunger/satiety peptides

Ghrelin, the only hunger-stimulating hormone, did not change (% change: CT=21.8 ± 49.2 % vs. WM=32.1 ± 103.8%) (Figure 8). There was a significant main effect of time to increase leptin (% change=8.6 ± 21.9%, p=0.021) (Figure 9). PYY increased via a time-by-treatment interaction in the control group only (% change = 23 ± 32%, p=0.001) (Figure 10). The effect size was medium (d=0.532). The incretin hormones GLP-1 and GIP varied in their response. There was a main effect of time to increase GLP-1 (% change=169.8 ± 531.0%, p=0.037) (Figure 11), while GIP did not significantly change (% change: CT=30.8 ± 83.9% vs WM=26.2 ± 130.0%) (Figure 12).

Chapter 5: Discussion

Over the course of the six-week treatment period, there was an overall main effect of time, which led to changes in body characteristics, specifically, weight and body fat percentage, with mass increasing significantly less than 1% and body fat percentage increasing approximately 1%. It is possible that the short duration of the study limited the impact on the changes observed. Fructose has been reported to increase body weight, total fat and visceral fat in humans consuming fructose-sweetened beverages (Stanhope et al., 2009), however, our treatment group did not increase their weight or body fat percentage independent of our control group. Therefore watermelon supplementation did not affect body characteristics.

As hypothesized, watermelon consumption increased plasma L-arginine in the watermelon group. Plasma L-citrulline, however, did not increase due to watermelon supplementation. These results are supported by the findings of Collins et al. that measured an increase in L-arginine but not L-citrulline with watermelon consumption (Collins et al., 2007). This is due to L-citrulline being utilized in the synthesis of L-arginine in order to produce NO (Wu & Morris, 1998).

Despite being within normal blood glucose ranges, Nichols et al. reported individuals who have blood glucose levels of 90-94mg/dl are at almost at a 50% greater risk of developing diabetes, compared to blood glucose levels less than 85mg/dl (Nichols et al., 2008). Both the control and watermelon groups had an average blood glucose level of at

least 95mg/dl, indicating that the subject population is predisposed to insulin resistance and is at risk of being diagnosed with impaired glucose tolerance. Fasting blood glucose levels did not change over time in either group. This indicates that within the six-week watermelon supplementation period, the dosage used neither improved nor impaired blood glucose homeostasis. Insulin levels less than 12µIU/mL are considered normal (Sinatra, 2004). Insulin increased as a result of time ($p=0.05$), increasing from 11µIU/mL to13µIU/mL. However, there was no difference due to treatment, suggesting that watermelon supplementation at the levels used neither improves nor makes insulin resistance worse within this population. These results support those reported by Wu et al. Zucker diabetic fatty rats did not change insulin levels due to watermelon supplementation (Wu et al., 2007). Although there was a trend, HOMA-IR did not significantly increase over time in the current study. This is consistent with the glucose findings. Our data suggest that while watermelon may not have improved insulin sensitivity, it also did not exacerbate insulin resistance. If we had found decreases in HOMA-IR, they would indicate improvements in insulin sensitivity and improved glucose.

Other studies have investigated the effect of L-arginine supplementation on hunger and satiety signaling (Batterham et al., 2003; Breitman et al., 2011; Broglio et al., 2004; Flatt, Kwasowski, Howland, & Bailey, 1991; Geloneze et al., 2014; Kaminski et al., 2015; Martin et al., 2014; Prodam et al., 2012; N. N. Rudovich et al., 2005; Tan et al., 2011). However, this is the first study designed to investigate the effects of watermelon supplementation on hunger and satiety peptides. Similar to other L-arginine supplementation studies in humans

(Breitman et al., 2011; Broglio et al., 2004; N. N. Rudovich et al., 2005), there was no change in ghrelin levels due to watermelon supplementation.

GIP did not change, which is similar to previous work. Breitman et al. reported no change in GIP over the course of 2 or 8 weeks of amino acid supplementation, with 7g of L-arginine as one of the components in their supplement (Breitman et al., 2011). However, our results do not agree with Flatt et al., who found an increase in GIP in obese, hyperglycemic mice when measured within 120 minutes of amino acid supplementation (Flatt et al., 1991). GIP is secreted in response to food intake. Therefore, it is possible that the time of the blood draw needs to be closer to time of consumption of watermelon in order to observe differences in GIP (Irwin & Flatt, 2009; Rudovich, Kaiser, Engeli, Osterhoff, Gogebakan, et al., 2007).

There was a main effect of time to increase GLP-1, but there was no effect of watermelon, which supports the findings of Breitman et al. that L-arginine supplementation did not change GLP-1 levels in humans (Breitman et al., 2011). The main effect of time may be due to changes in diet. All subjects were instructed to avoid consumption of L-arginine rich foods, therefore, GLP-1 levels may have declined, since it is secreted in response to proteins. Also, GLP-1 is released 10-15 and 30-60 minutes after oral ingestion of food in a biphasic pattern, therefore, levels may have returned to baseline by the time of the blood draw.

Leptin did not independently change in our watermelon treatment group, which contradicts previous studies that have reported increases in leptin due to amino acid

supplementation that contains L-arginine supplementation (Breitman et al., 2011; Tan et al., 2011). There was a main effect of time in leptin concentrations over the six weeks. This could be a reflection of the increase in adipose tissue also observed.

PYY was reported to increase in the control group with a medium effect size compared to watermelon supplementation. PYY has been reported to be secreted due to food intake, specifically carbohydrates and lipids (Thomas & Schauer, 2010). This study was conducted in a community setting, so the increase in PYY could be due to dietary choices made within the control group.

Lack of dietary control could be considered a limitation of the study. In a community setting study, food choices are not controlled. The instruction was to limit L-arginine consumption. Changes in diet may have increased variability of the results. If their new diet was composed of more carbohydrates, there may have been a need to increase insulin to maintain the blood glucose levels. Requiring an increase in insulin could lead to an increase of incretin hormones, such as GLP-1, which occurred in both groups.

Our supplementation program required the watermelon treatment group to consume 710mls of watermelon puree every day, but the subjects were allowed to decide on time of consumption and if they would consume it all at once or in smaller doses. Therefore, the watermelon group could have consumed varying amounts of watermelon puree at different time points throughout the day. As a result, some of the variables we measured could have peaked at different times based on the last time the watermelon was consumed. Another

limitation is only collecting fasting blood draws. Some of the hunger and satiety peptides have a relatively short half-life; therefore, by only taking fasting blood draws, we may not have observed the physiological changes as a result of watermelon supplementation.

In future studies, either standardizing the diet or not restricting L-arginine rich foods could be beneficial. A standardized diet would offer stronger comparisons between groups, while a non-restricted diet would be the more applicable for a community-based methodology. Also, sample collection at various time points after the last consumption of watermelon might be useful for detecting changes in signaling molecules that degrade quickly. The addition of a physiological stress, such as exercise, in addition to watermelon supplementation, could allow for determination of a potential benefit of watermelon.

In conclusion, watermelon supplementation did not significantly impact hunger and satiety signaling peptides in overweight, post-menopausal women. Glucose, insulin and HOMA-IR were also not affected by watermelon supplementation. Plasma L-arginine increased as a result of watermelon supplementation, thus watermelon could be utilized to supplement individuals with low L-arginine concentrations. Therefore, if watermelon supplementation was utilized for therapeutic purposes, it would not result in an alteration of their insulin sensitivity or food intake signaling.

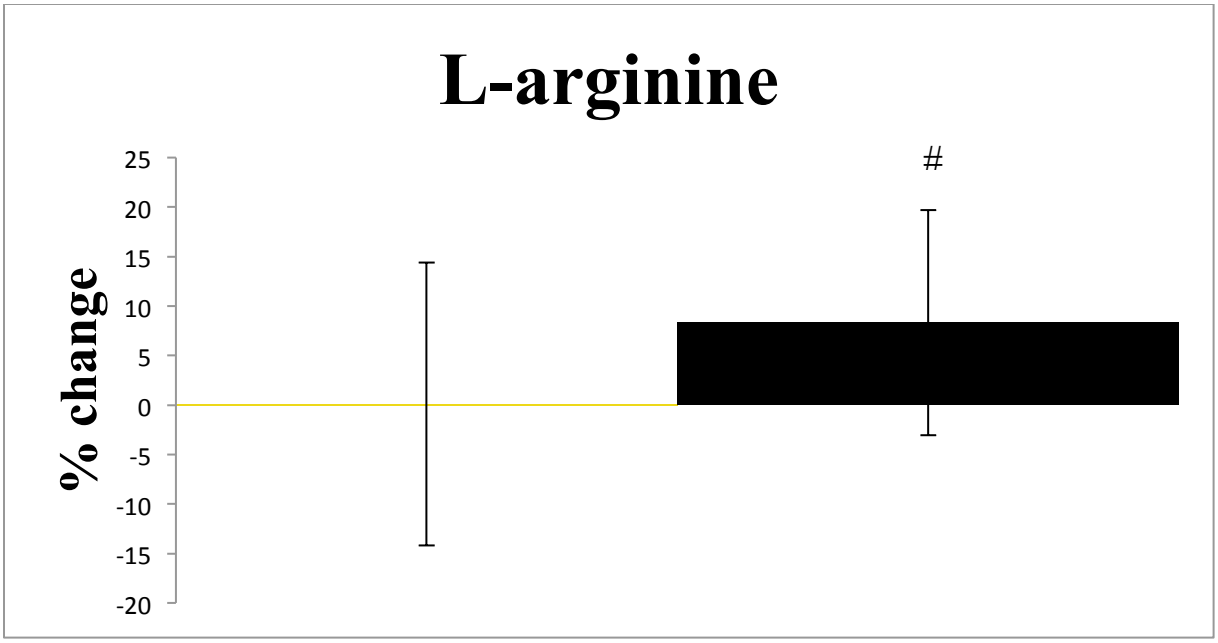

Figure 3. L-rginine change in the CT and WM groups (% change CT 0.1 ± 14.3% (% change WM 8.3 ± 11.4%)). Time-by-treatment interaction, #p<0.05.

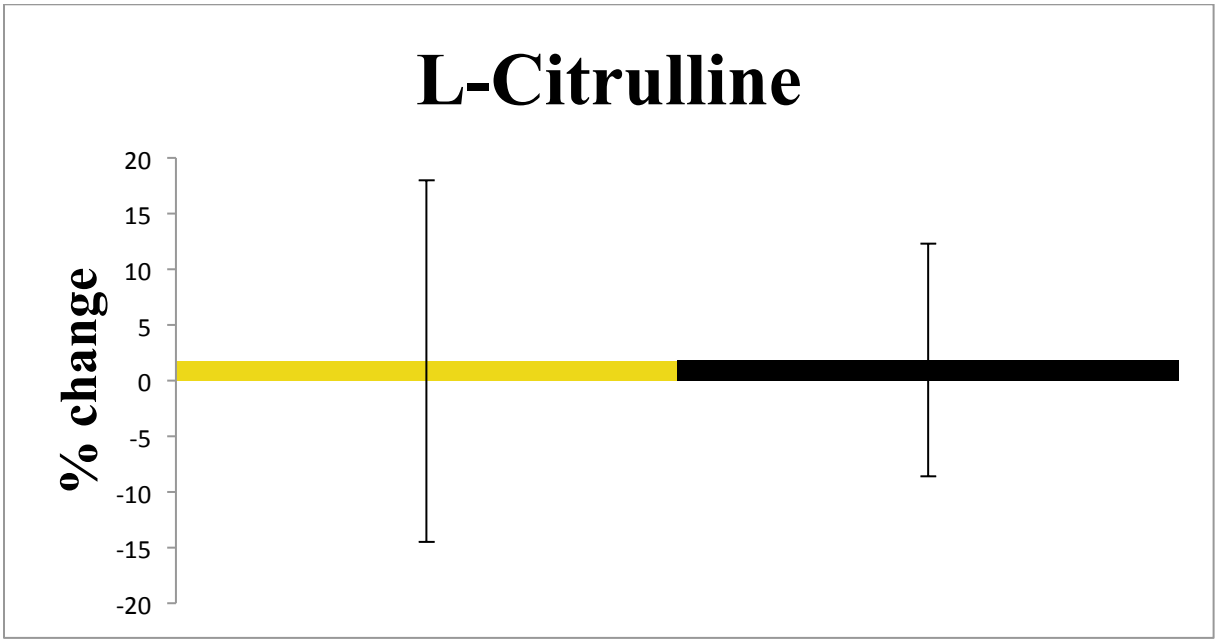

Figure 4. L-citrulline change in the CT and WM groups (% change CT 1.7 ± 16.2%, % change WM 1.8 ± 10.4%).

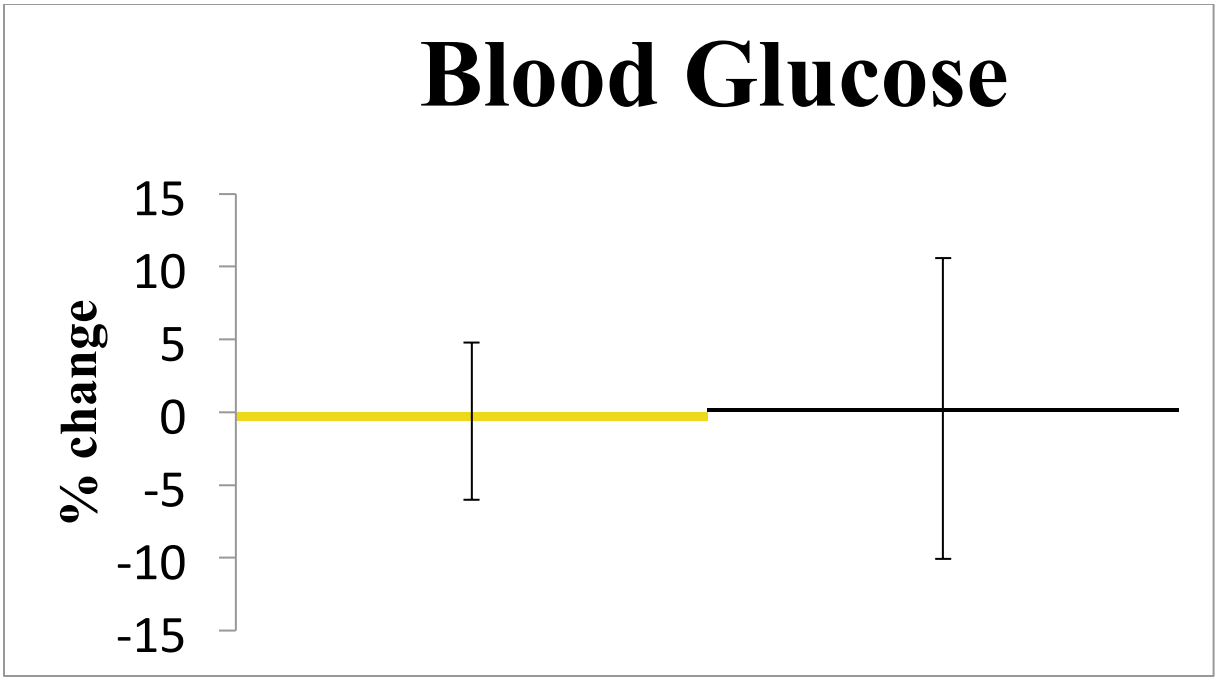

Figure 5. Blood glucose change in the CT and WM groups (% change CT-0.6 ± 5.4% std, % change WM 0.26 ± 6.8%).

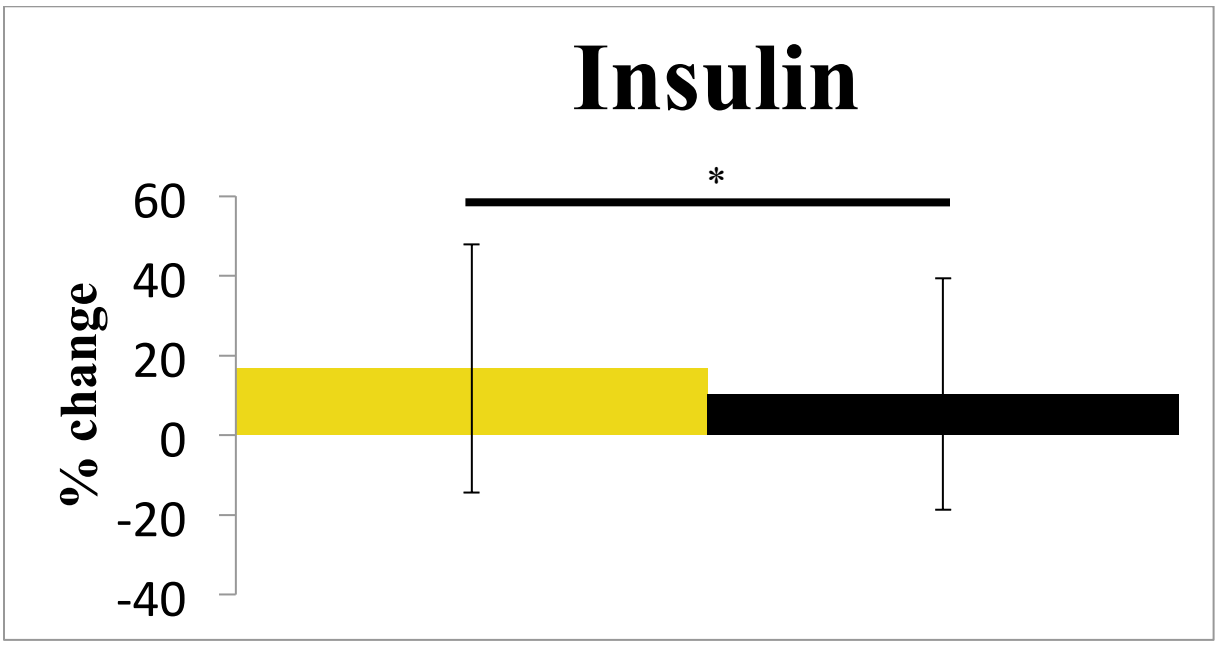

Figure 6. Insulin change in the CT and WM groups (% change CT 16.8 ± 31.2%, % change WM 10.3 ± 29.0%). Main effect of time (13.0 ± 30.1%) *p<0.05.

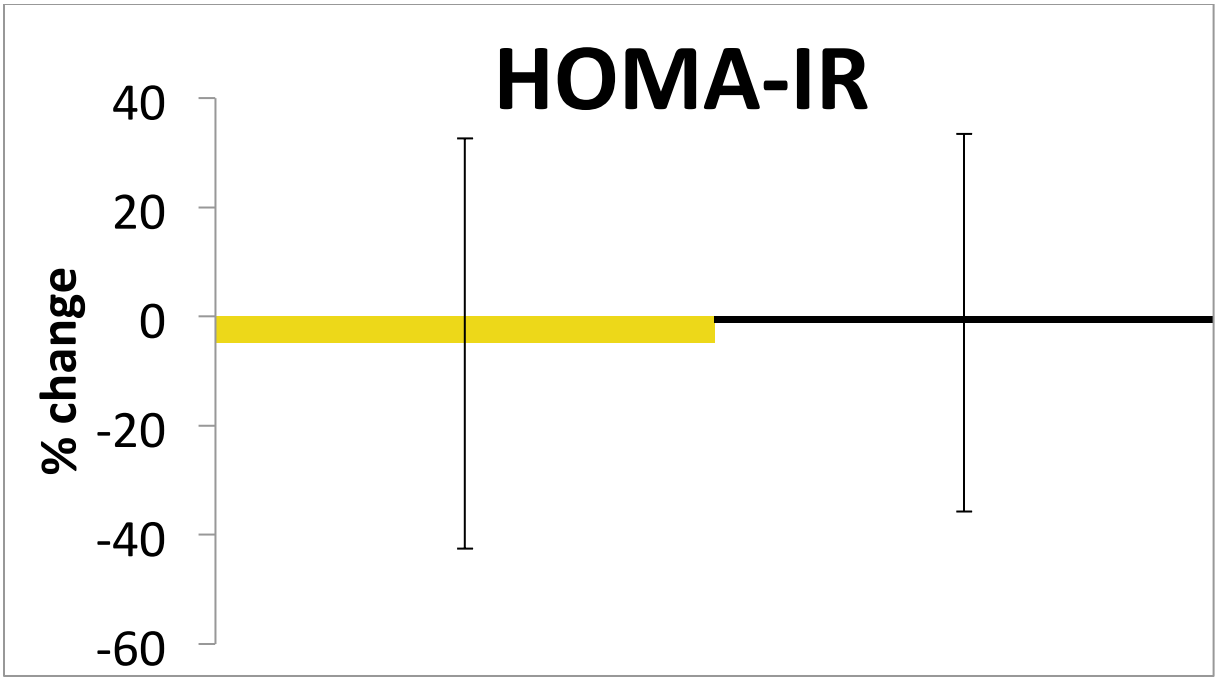

Figure 7. HOMA-IR, homeostatic model assessment of insulin resistance change in the CT and WM groups (% change CT -4.9 ± 37.6%, % change WM -1.2 ± 34.6%).

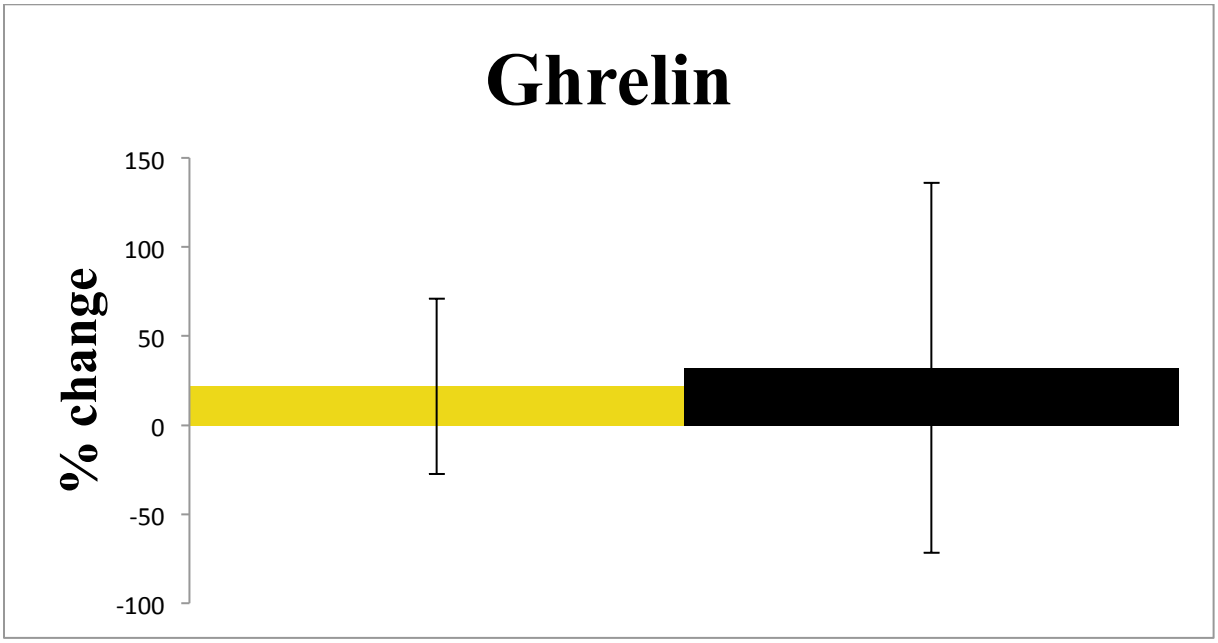

Figure 8. Ghrelin change in the CT and WM groups (% change CT 21.8 ± 49.2%, % change WM 32.1 ± 103.8%).

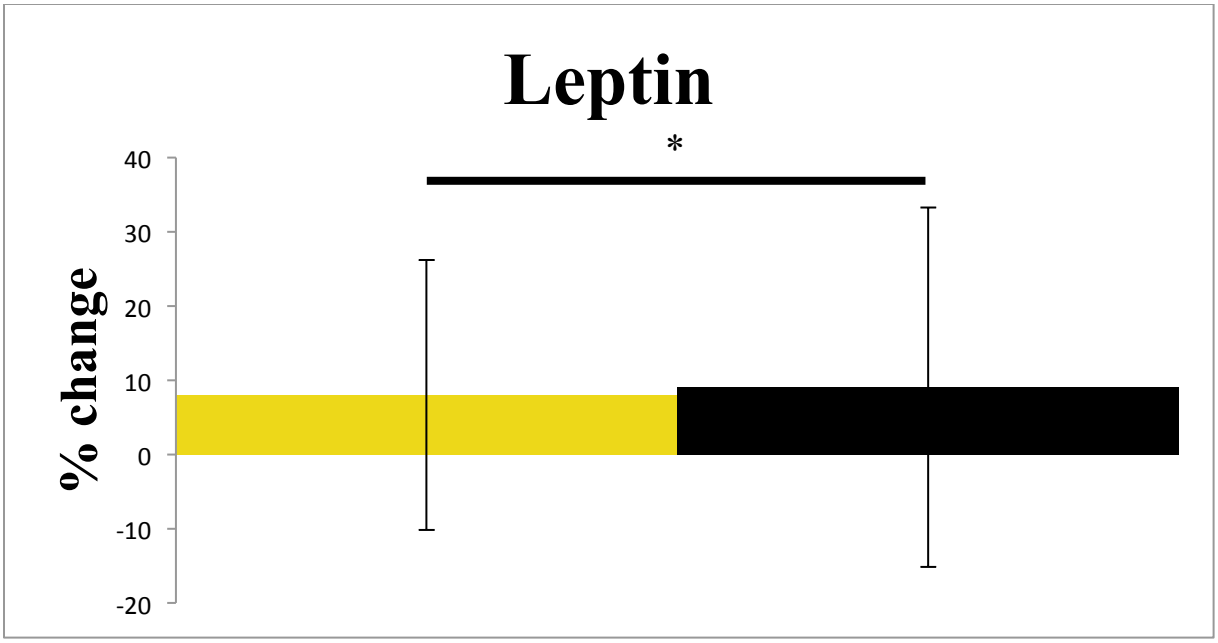

Figure 9**.** Leptin change in the CT and WM groups (% change CT 8.0 ± 18.2%, % change WM 9.1 ± 24.2%). Main effect of time (8.6 ± 21.9%), *p<0.05.

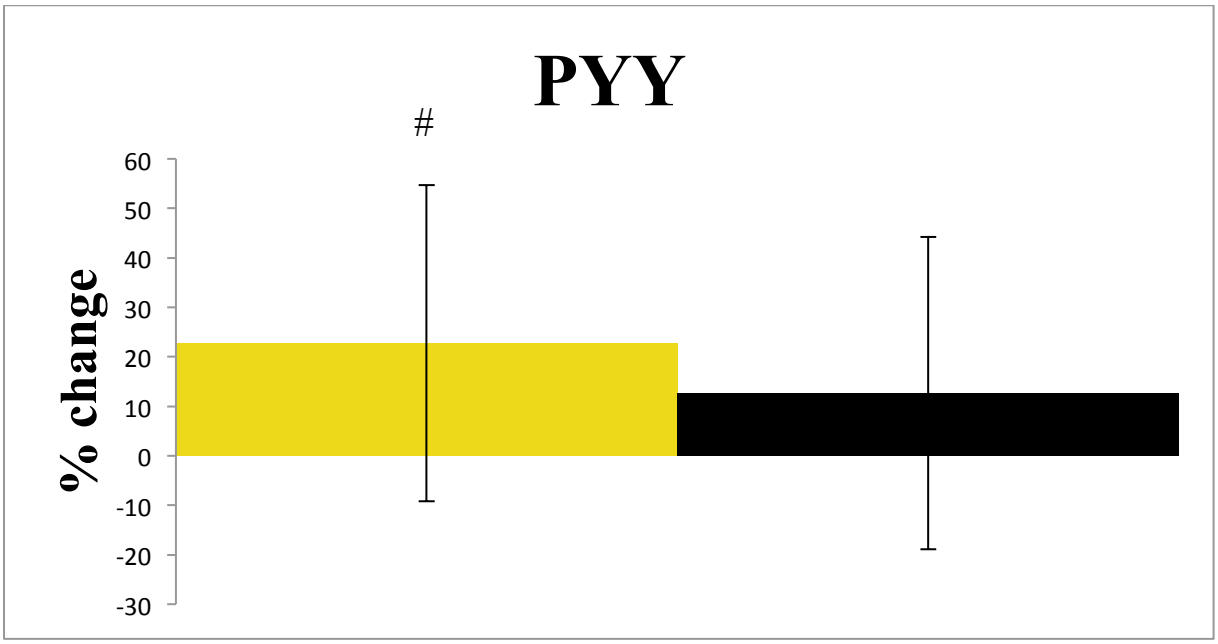

Figure 10. PYY change in the CT and WM groups (% change CT 22.7 ± 32.0%, % change WM 12.7 ± 31.6%). Time-by-treatment interaction, #p<0.05.

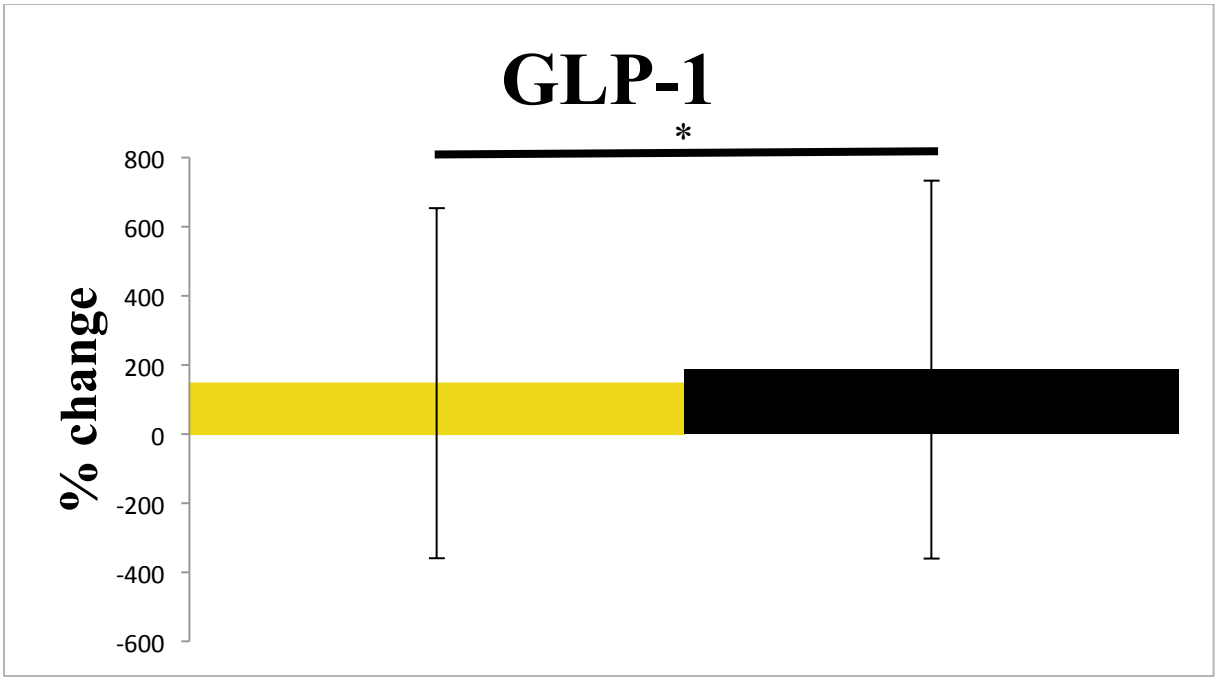

Figure 11. GLP-1change in the CT and WM groups (% change CT 146.3 ± 506.7%, % change WM 186.9 ± 547.4%). Main effect of time (169.8 ± 530.9%), *p<0.05

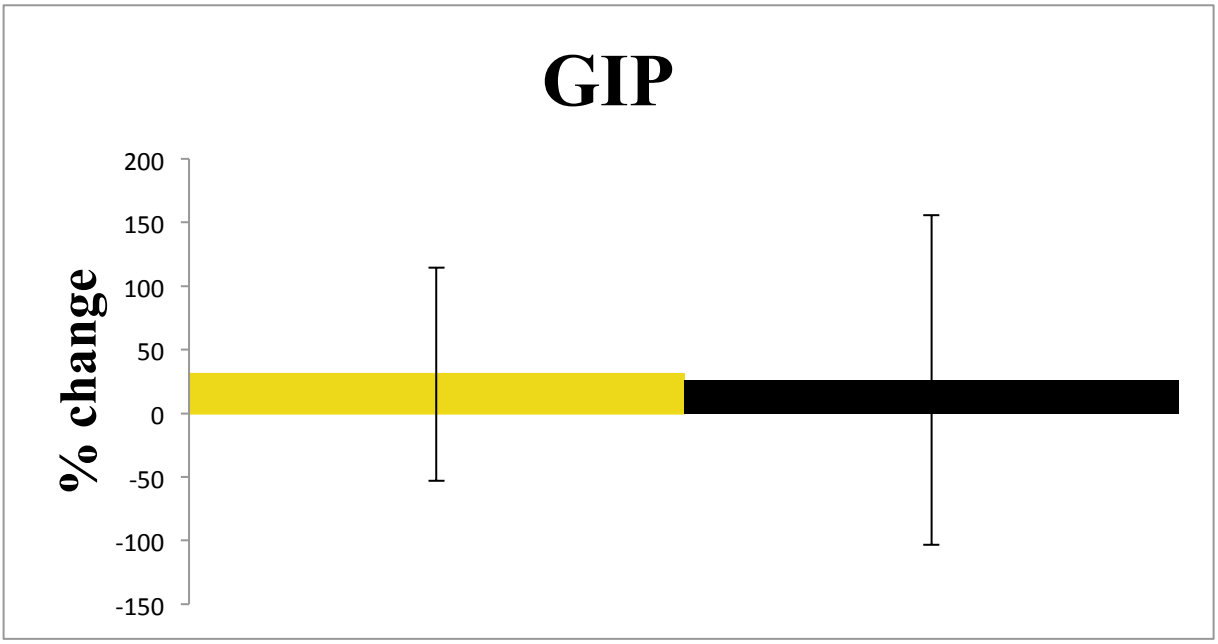

Figure 12. GIP change in the CT and WM groups (% change CT 30.8 ± 83.9%, % change WM 26.2 ± 129.7%).

References

Abdul Razak, H. R., & Saad, W. M. *Biomed Res Int*. doi: 10.1155/2014/512834

Adamska, E., Ostrowska, L., Gorska, M., & Kretowski, A. (2014). The role of gastrointestinal hormones in the pathogenesis of obesity and type 2 diabetes. *Prz Gastroenterol, 9*(2), 69-76. doi: 10.5114/pg.2014.42498

Adrian, T. E., Ferri, G. L., Bacarese-Hamilton, A. J., Fuessl, H. S., Polak, J. M., & Bloom, S. R. (1985). Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology, 89*(5), 1070-1077.

Ahima, R. S., & Osei, S. Y. (2004). Leptin signaling. *Physiol Behav, 81*(2), 223-241. doi: 10.1016/j.physbeh.2004.02.014

Al-Jiffri, O., Al-Sharif, F. M., Abd El-Kader, S. M., & Ashmawy, E. M. (2013). Weight reduction improves markers of hepatic function and insulin resistance in type-2 diabetic patients with non-alcoholic fatty liver. *Afr Health Sci, 13*(3), 667-672. doi: 10.4314/ahs.v13i3.21

Al-Safi, Z. A., & Polotsky, A. J. (2014). Obesity and Menopause. *Best Pract Res Clin Obstet Gynaecol*. doi: 10.1016/j.bpobgyn.2014.12.002

Batterham, R. L., Cohen, M. A., Ellis, S. M., Le Roux, C. W., Withers, D. J., Frost, G. S., . . . Bloom, S. R. (2003). Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J Med, 349*(10), 941-948. doi: 10.1056/NEJMoa030204

Boger, R. H. (2014). The pharmacodynamics of L-arginine. *Altern Ther Health Med, 20*(3), 48-54.

Breitman, I., Saraf, N., Kakade, M., Yellumahanthi, K., White, M., Hackett, J. A., & Clements, R. H. (2011). The effects of an amino acid supplement on glucose homeostasis, inflammatory markers, and incretins after laparoscopic gastric bypass. *J Am Coll Surg, 212*(4), 617-625; discussion 625-617. doi: 10.1016/j.jamcollsurg.2010.12.040

Broglio, F., Gottero, C., Prodam, F., Destefanis, S., Gauna, C., Me, E., . . . Ghigo, E. (2004). Ghrelin secretion is inhibited by glucose load and insulin-induced hypoglycaemia but unaffected by glucagon and arginine in humans. *Clin Endocrinol (Oxf), 61*(4), 503-509. doi: 10.1111/j.1365-2265.2004.02121.x

Butera, P. C. (2010). Estradiol and the control of food intake. *Physiol Behav, 99*(2), 175-180. doi: 10.1016/j.physbeh.2009.06.010

Cahill, F., Ji, Y., Wadden, D., Amini, P., Randell, E., Vasdev, S., . . . Sun, G. (2014). The Association of Serum Total Peptide YY (PYY) with Obesity and Body Fat Measures in the CODING Study. *PLoS One, 9*(4), e95235. doi: 10.1371/journal.pone.0095235

Calapai, G., Corica, F., Allegra, A., Corsonello, A., Sautebin, L., De Gregorio, T., . . . Caputi, A. P. (1998). Effects of intracerebroventricular leptin administration on food intake, body weight gain and diencephalic nitric oxide synthase activity in the mouse. *Br J Pharmacol, 125*(4), 798-802. doi: 10.1038/sj.bjp.0702121

Carvalho-Filho, M. A., Ueno, M., Carvalheira, J. B., Velloso, L. A., & Saad, M. J. (2006). Targeted disruption of iNOS prevents LPS-induced S-nitrosation of IRbeta/IRS-1 and Akt and insulin resistance in muscle of mice. *Am J Physiol Endocrinol Metab, 291*(3), E476-482. doi: 10.1152/ajpendo.00422.2005

Carvalho-Filho, M. A., Ueno, M., Hirabara, S. M., Seabra, A. B., Carvalheira, J. B., de Oliveira, M. G., . . . Saad, M. J. (2005). S-nitrosation of the insulin receptor, insulin receptor substrate 1, and protein kinase B/Akt: a novel mechanism of insulin resistance. *Diabetes, 54*(4), 959-967.

Castillo, L., Chapman, T. E., Yu, Y. M., Ajami, A., Burke, J. F., & Young, V. R. (1993). Dietary arginine uptake by the splanchnic region in adult humans. *Am J Physiol, 265*(4 Pt 1), E532-539.

Cersosimo, E., & DeFronzo, R. A. (2006). Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. *Diabetes Metab Res Rev, 22*(6), 423-436. doi: 10.1002/dmrr.634

Cervellati, C., Pansini, F. S., Bonaccorsi, G., Pascale, G., Bagni, B., Castaldini, C., . . . Bergamini, C. M. (2009). Body mass index is a major determinant of abdominal fat accumulation in pre-, peri- and post-menopausal women. *Gynecol Endocrinol, 25*(6), 413-417.

Chedraui, P., Perez-Lopez, F. R., Escobar, G. S., Palla, G., Montt-Guevara, M., Cecchi, E., . . . Simoncini, T. (2014). Circulating leptin, resistin, adiponectin, visfatin, adipsin and ghrelin levels and insulin resistance in postmenopausal women with and without the metabolic syndrome. *Maturitas, 79*(1), 86-90. doi: 10.1016/j.maturitas.2014.06.008

Chiang, Y. T., Ip, W., & Jin, T. (2012). The role of the Wnt signaling pathway in incretin hormone production and function. *Front Physiol, 3*, 273. doi: 10.3389/fphys.2012.00273

Cohen, J. (1988). *Statistical power analysis of the behavioral sciences (2nd ed.)*. Hilsdale, NJ: Lawrence Earlbaum Associates.

Collins, J. K., Wu, G., Perkins-Veazie, P., Spears, K., Claypool, P. L., Baker, R. A., & Clevidence, B. A. (2007). Watermelon consumption increases plasma arginine concentrations in adults. *Nutrition, 23*(3), 261-266. doi: 10.1016/j.nut.2007.01.005

Dalamaga, M., Chou, S. H., Shields, K., Papageorgiou, P., Polyzos, S. A., & Mantzoros, C. S. (2013). Leptin at the intersection of neuroendocrinology and metabolism: current evidence and therapeutic perspectives. *Cell Metab, 18*(1), 29-42. doi: 10.1016/j.cmet.2013.05.010

Deibert, P., Konig, D., Vitolins, M. Z., Landmann, U., Frey, I., Zahradnik, H. P., & Berg, A. (2007). Effect of a weight loss intervention on anthropometric measures and metabolic risk factors in pre- versus postmenopausal women. *Nutr J, 6*, 31. doi: 10.1186/1475-2891-6-31

Duplain, H., Burcelin, R., Sartori, C., Cook, S., Egli, M., Lepori, M., . . . Scherrer, U. (2001). Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation, 104*(3), 342-345.

El-Haschimi, K., Pierroz, D. D., Hileman, S. M., Bjorbaek, C., & Flier, J. S. (2000). Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest, 105*(12), 1827-1832. doi: 10.1172/jci9842

Elliott, R. M., Morgan, L. M., Tredger, J. A., Deacon, S., Wright, J., & Marks, V. (1993). Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol, 138*(1), 159-166.

Eshtiaghi, R., Esteghamati, A., & Nakhjavani, M. (2010). Menopause is an independent predictor of metabolic syndrome in Iranian women. *Maturitas, 65*(3), 262-266. doi: 10.1016/j.maturitas.2009.11.004

Essah, P. A., Levy, J. R., Sistrun, S. N., Kelly, S. M., & Nestler, J. E. (2007). Effect of macronutrient composition on postprandial peptide YY levels. *J Clin Endocrinol Metab, 92*(10), 4052-4055. doi: 10.1210/jc.2006-2273

Ezcurra, M., Reimann, F., Gribble, F. M., & Emery, E. (2013). Molecular mechanisms of incretin hormone secretion. *Curr Opin Pharmacol, 13*(6), 922-927. doi: 10.1016/j.coph.2013.08.013

Farr, S. A., Banks, W. A., Kumar, V. B., & Morley, J. E. (2005). Orexin-A-induced feeding is dependent on nitric oxide. *Peptides, 26*(5), 759-765. doi: 10.1016/j.peptides.2004.12.004

Figueroa, A., Sanchez-Gonzalez, M. A., Perkins-Veazie, P. M., & Arjmandi, B. H. (2011). Effects of watermelon supplementation on aortic blood pressure and wave reflection in individuals with prehypertension: a pilot study. *Am J Hypertens, 24*(1), 40-44. doi: 10.1038/ajh.2010.142

Figueroa, A., Sanchez-Gonzalez, M. A., Wong, A., & Arjmandi, B. H. (2012). Watermelon extract supplementation reduces ankle blood pressure and carotid augmentation index in obese adults with prehypertension or hypertension. *Am J Hypertens, 25*(6), 640-643. doi: 10.1038/ajh.2012.20

Figueroa, A., Wong, A., Hooshmand, S., & Sanchez-Gonzalez, M. A. (2013). Effects of watermelon supplementation on arterial stiffness and wave reflection amplitude in postmenopausal women. *Menopause, 20*(5), 573-577. doi: 10.1097/GME.0b013e3182733794

Figueroa, A., Wong, A., & Kalfon, R. (2014). Effects of watermelon supplementation on aortic hemodynamic responses to the cold pressor test in obese hypertensive adults. *Am J Hypertens, 27*(7), 899-906. doi: 10.1093/ajh/hpt295

Flatt, P. R., Kwasowski, P., Howland, R. J., & Bailey, C. J. (1991). Gastric inhibitory polypeptide and insulin responses to orally administered amino acids in genetically obese hyperglycemic (ob/ob) mice. *J Nutr, 121*(7), 1123-1128.

Gaskin, F. S., Farr, S. A., Banks, W. A., Kumar, V. B., & Morley, J. E. (2003). Ghrelin-induced feeding is dependent on nitric oxide. *Peptides, 24*(6), 913-918.

Gault, V. A., O'Harte, F. P., & Flatt, P. R. (2003). Glucose-dependent insulinotropic polypeptide (GIP): anti-diabetic and anti-obesity potential? *Neuropeptides, 37*(5), 253-263.

Geloneze, B., de Oliveira Mda, S., Vasques, A. C., Novaes, F. S., Pareja, J. C., & Tambascia, M. A. (2014). Impaired incretin secretion and pancreatic dysfunction with older age and diabetes. *Metabolism, 63*(7), 922-929. doi: 10.1016/j.metabol.2014.04.004

Gentilcore, D., Visvanathan, R., Russo, A., Chaikomin, R., Stevens, J. E., Wishart, J. M., . . . Jones, K. L. (2005). Role of nitric oxide mechanisms in gastric emptying of, and the blood pressure and glycemic responses to, oral glucose in healthy older subjects. *Am J Physiol Gastrointest Liver Physiol, 288*(6), G1227-1232. doi: 10.1152/ajpgi.00511.2004

Gibbons, C., Caudwell, P., Finlayson, G., Webb, D. L., Hellstrom, P. M., Naslund, E., & Blundell, J. E. (2013). Comparison of postprandial profiles of ghrelin, active GLP-1, and total PYY to meals varying in fat and carbohydrate and their association with hunger and the phases of satiety. *J Clin Endocrinol Metab, 98*(5), E847-855. doi: 10.1210/jc.2012-3835

Hermans, M. P., Levy, J. C., Morris, R. J., & Turner, R. C. (1999). Comparison of insulin sensitivity tests across a range of glucose tolerance from normal to diabetes. *Diabetologia, 42*(6), 678-687. doi: 10.1007/s001250051215

Hill, B. R., De Souza, M. J., Wagstaff, D. A., Sato, R., & Williams, N. I. (2012). 24-hour profiles of circulating ghrelin and peptide YY are inversely associated in normal weight premenopausal women. *Peptides, 38*(1), 159-162. doi: 10.1016/j.peptides.2012.08.017

Holowatz, L. A., Thompson-Torgerson, C., & Kenney, W. L. (2010). Aging and the control of human skin blood flow. *Front Biosci (Landmark Ed), 15*, 718-739.

Irwin, N., & Flatt, P. R. (2009). Evidence for beneficial effects of compromised gastric inhibitory polypeptide action in obesity-related diabetes and possible therapeutic implications. *Diabetologia, 52*(9), 1724-1731. doi: 10.1007/s00125-009-1422-8

Irwin, N., & Flatt, P. R. (2013). Enteroendocrine hormone mimetics for the treatment of obesity and diabetes. *Curr Opin Pharmacol, 13*(6), 989-995. doi: 10.1016/j.coph.2013.09.009

Jobgen, W., Meininger, C. J., Jobgen, S. C., Li, P., Lee, M. J., Smith, S. B., . . . Wu, G. (2009). Dietary L-arginine supplementation reduces white fat gain and enhances skeletal muscle and brown fat masses in diet-induced obese rats. *J Nutr, 139*(2), 230-237. doi: 10.3945/jn.108.096362

Joffin, N., Niang, F., Forest, C., & Jaubert, A. M. (2012). Is there NO help for leptin? *Biochimie, 94*(10), 2104-2110. doi: 10.1016/j.biochi.2012.06.017

Kaminski, S. L., Redmer, D. A., Bass, C. S., Keisler, D. H., Carlson, L. S., Vonnahme, K. A., . . . Grazul-Bilska, A. T. (2015). The effects of diet and arginine treatment on serum metabolites and selected hormones during the estrous cycle in sheep. *Theriogenology, 83*(5), 808-816. doi: 10.1016/j.theriogenology.2014.11.017

Katusic, Z. S. (2001). Vascular endothelial dysfunction: does tetrahydrobiopterin play a role? *Am J Physiol Heart Circ Physiol, 281*(3), H981-986.

Keung, W., Palaniyappan, A., & Lopaschuk, G. D. (2011). Chronic central leptin decreases food intake and improves glucose tolerance in diet-induced obese mice independent of hypothalamic malonyl CoA levels and skeletal muscle insulin sensitivity. *Endocrinology, 152*(11), 4127-4137. doi: 10.1210/en.2011-1254

Kim, & Egan, J. M. (2008). The role of incretins in glucose homeostasis and diabetes treatment. *Pharmacol Rev, 60*(4), 470-512. doi: 10.1124/pr.108.000604

Kim, & Feldman. (2015). Insulin resistance as a key link for the increased risk of cognitive impairment in the metabolic syndrome. *Exp Mol Med, 47*, e149. doi: 10.1038/emm.2015.3

Klok, M. D., Jakobsdottir, S., & Drent, M. L. (2007). The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. *Obes Rev, 8*(1), 21-34. doi: 10.1111/j.1467-789X.2006.00270.x

Le, K. A., & Tappy, L. (2006). Metabolic effects of fructose. *Curr Opin Clin Nutr Metab Care, 9*(4), 469-475. doi: 10.1097/01.mco.0000232910.61612.4d

Lee, & Jun, H. S. (2014). Anti-diabetic actions of glucagon-like peptide-1 on pancreatic beta-cells. *Metabolism, 63*(1), 9-19. doi: 10.1016/j.metabol.2013.09.010

Lee, S. W., Jo, H. H., Kim, M. R., Kim, J. H., & You, Y. O. (2015). Association between osteocalcin and metabolic syndrome in postmenopausal women. *Arch Gynecol Obstet*. doi: 10.1007/s00404-015-3656-7

Leshan, R. L., Bjornholm, M., Munzberg, H., & Myers, M. G., Jr. (2006). Leptin receptor signaling and action in the central nervous system. *Obesity (Silver Spring), 14 Suppl 5*, 208S-212S. doi: 10.1038/oby.2006.310

Ley, C. J., Lees, B., & Stevenson, J. C. (1992). Sex- and menopause-associated changes in body-fat distribution. *Am J Clin Nutr, 55*(5), 950-954.

Lizarbe, B., Benitez, A., Pelaez Brioso, G. A., Sanchez-Montanes, M., Lopez-Larrubia, P., Ballesteros, P., & Cerdan, S. (2013). Hypothalamic metabolic compartmentation during appetite regulation as revealed by magnetic resonance imaging and spectroscopy methods. *Front Neuroenergetics, 5*, 6. doi: 10.3389/fnene.2013.00006

Lizcano, F., & Guzman, G. (2014). Estrogen Deficiency and the Origin of Obesity during Menopause. *Biomed Res Int, 2014*, 757461. doi: 10.1155/2014/757461

Lustig, R. H. (2010). Fructose: metabolic, hedonic, and societal parallels with ethanol. *Journal of the American Dietetic Association, 110*(9), 1307-1321. doi: 10.1016/j.jada.2010.06.008

Martin, L. J., Lutz, T. A., Daumas, C., Bleis, P., Nguyen, P., Biourge, V., & Dumon, H. J. (2014). Acute hormonal response to glucose, lipids and arginine infusion in overweight cats. *J Nutr Sci, 3*, e8. doi: 10.1017/jns.2014.4

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*(7), 412-419.

MayoClinic. (2015). Menopause. Retrieved 4/10, 2015, from http://www.mayoclinic.org/diseases-conditions/menopause/basics/definition/con-20019726

Mehebik-Mojaat, N., Ribiere, C., Niang, F., Forest, C., & Jaubert, A. M. (2009). Leptin and insulin induce mutual resistance for nitric oxide synthase III activation in adipocytes. *J Cell Biochem, 108*(4), 982-988. doi: 10.1002/jcb.22331

Mohammad, M. K., Mohamed, M. I., & Zakaria, A. M. (2014). Watermelon (Citrullus lanatus (Thunb.) Matsum. and Nakai) juice modulates oxidative damage induced by low dose X-ray in mice. *2014*, 512834. doi: 10.1155/2014/512834

Mohammad, M. K., Mohamed, M. I., Zakaria, A. M., Abdul Razak, H. R., & Saad, W. M. (2014). Watermelon (Citrullus lanatus (Thunb.) Matsum. and Nakai) juice modulates oxidative damage induced by low dose X-ray in mice. *Biomed Res Int, 2014*, 512834. doi: 10.1155/2014/512834

Moon, H. S., Dalamaga, M., Kim, S. Y., Polyzos, S. A., Hamnvik, O. P., Magkos, F., . . . Mantzoros, C. S. (2013). Leptin's role in lipodystrophic and nonlipodystrophic

insulin-resistant and diabetic individuals. *Endocr Rev, 34*(3), 377-412. doi: 10.1210/er.2012-1053

Moran, T. H. (2009). Fructose and satiety. *J Nutr, 139*(6), 1253s-1256s. doi: 10.3945/jn.108.097956

Morley, J. E., Alshaher, M. M., Farr, S. A., Flood, J. F., & Kumar, V. B. (1999). Leptin and neuropeptide Y (NPY) modulate nitric oxide synthase: further evidence for a role of nitric oxide in feeding. *Peptides, 20*(5), 595-600.

Morley, J. E., Farr, S. A., Sell, R. L., Hileman, S. M., & Banks, W. A. (2011). Nitric oxide is a central component in neuropeptide regulation of appetite. *Peptides, 32*(4), 776-780. doi: 10.1016/j.peptides.2010.12.015

Nagai, Y., Yonemitsu, S., Erion, D. M., Iwasaki, T., Stark, R., Weismann, D., . . . Löffler, M. G. (2009). The role of peroxisome proliferator-activated receptor γ coactivator-1 β in the pathogenesis of fructose-induced insulin resistance. *Cell Metab, 9*(3), 252-264.

Nichols, G. A., Hillier, T. A., & Brown, J. B. (2008). Normal fasting plasma glucose and risk of type 2 diabetes diagnosis. *Am J Med, 121*(6), 519-524. doi: 10.1016/j.amjmed.2008.02.026

Ostlund, R. E., Jr., Yang, J. W., Klein, S., & Gingerich, R. (1996). Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab, 81*(11), 3909-3913. doi: 10.1210/jcem.81.11.8923837

Oyinloye, B. E., Adenowo, A. F., & Kappo, A. P. (2015). Reactive oxygen species, apoptosis, antimicrobial peptides and human inflammatory diseases. *Pharmaceuticals (Basel), 8*(2), 151-175. doi: 10.3390/ph8020151

Paneni, F., Costantino, S., & Cosentino, F. (2015). Role of oxidative stress in endothelial insulin resistance. *World J Diabetes, 6*(2), 326-332. doi: 10.4239/wjd.v6.i2.326

Park, H. K., & Ahima, R. S. (2014). Leptin signaling. *F1000Prime Rep, 6*, 73. doi: 10.12703/p6-73

Paz-Filho, G., Mastronardi, C., Franco, C. B., Wang, K. B., Wong, M. L., & Licinio, J. (2012). Leptin: molecular mechanisms, systemic pro-inflammatory effects, and clinical implications. *Arq Bras Endocrinol Metabol, 56*(9), 597-607.

Pederson, R. A., & Brown, J. C. (1976). The insulinotropic action of gastric inhibitory polypeptide in the perfused isolated rat pancreas. *Endocrinology, 99*(3), 780-785. doi: 10.1210/endo-99-3-780

Perkins-Veazie, P., Collins, J. K., Pair, S. D., & Roberts, W. (2001). Lycopene content differs among red-fleshed watermelon cultivars. *Journal of the Science of Food and Agriculture, 81*(10), 983-987. doi: 10.1002/jsfa.880

Perreault, M., & Marette, A. (2001). Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat Med, 7*(10), 1138-1143. doi: 10.1038/nm1001-1138

Powers, S. K., Nelson, W. B., & Hudson, M. B. (2011). Exercise-induced oxidative stress in humans: cause and consequences. *Free Radic Biol Med, 51*(5), 942-950. doi: 10.1016/j.freeradbiomed.2010.12.009

Prodam, F., Genoni, G., Bellone, S., Longhi, S., Agarla, V., Bona, G., & Radetti, G. (2012). Effect of Arginine Infusion on Ghrelin Secretion in Growth Hormone Sufficient and GH Deficient Children. *Int J Endocrinol Metab, 10*(2), 470-474. doi: 10.5812/ijem.3826

Rocca, A. S., & Brubaker, P. L. (1999). Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology, 140*(4), 1687-1694. doi: 10.1210/endo.140.4.6643

Roe, N. D., & Ren, J. (2012). Nitric oxide synthase uncoupling: a therapeutic target in cardiovascular diseases. *Vascul Pharmacol, 57*(5-6), 168-172. doi: 10.1016/j.vph.2012.02.004

Ropelle, E. R., Pauli, J. R., Cintra, D. E., da Silva, A. S., De Souza, C. T., Guadagnini, D., . . . Carvalheira, J. B. (2013). Targeted disruption of inducible nitric oxide synthase protects against aging, S-nitrosation, and insulin resistance in muscle of male mice. *Diabetes, 62*(2), 466-470. doi: 10.2337/db12-0339

Rudovich, Kaiser, S., Engeli, S., Osterhoff, M., Gogebakan, O., Bluher, M., & Pfeiffer, A. F. (2007). GIP receptor mRNA expression in different fat tissue depots in postmenopausal non-diabetic women. *Regul Pept, 142*(3), 138-145. doi: 10.1016/j.regpep.2007.02.006

Rudovich, Kaiser, S., Engeli, S., Osterhoff, M., Gögebakan, Ö., Bluher, M., & Pfeiffer, A. F. H. (2007). GIP receptor mRNA expression in different fat tissue depots in

postmenopausal non-diabetic women. *Regul Pept, 142*(3), 138-145. doi: 10.1016/j.regpep.2007.02.006

Rudovich, N. N., Dick, D., Moehlig, M., Otto, B., Spranger, J., Rochlitz, H. J., . . . Pfeiffer, A. F. (2005). Ghrelin is not suppressed in hyperglycemic clamps by gastric inhibitory polypeptide and arginine. *Regul Pept, 127*(1-3), 95-99. doi: 10.1016/j.regpep.2004.10.012

Sainz, N., Barrenetxe, J., Moreno-Aliaga, M. J., & Martinez, J. A. (2015). Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. *Metabolism, 64*(1), 35-46. doi: 10.1016/j.metabol.2014.10.015

Saltiel, A. R., & Kahn, C. R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature, 414*(6865), 799-806. doi: 10.1038/414799a

Sansbury, B. E., & Hill, B. G. (2014). Regulation of obesity and insulin resistance by nitric oxide. *Free Radic Biol Med, 73c*, 383-399. doi: 10.1016/j.freeradbiomed.2014.05.016

Shaw, J. E., Zimmet, P. Z., McCarty, D., & de Courten, M. (2000). Type 2 diabetes worldwide according to the new classification and criteria. *Diabetes Care, 23 Suppl 2*, B5-10.

Shinozaki, S., Choi, C. S., Shimizu, N., Yamada, M., Kim, M., Zhang, T., . . . Kaneki, M. (2011). Liver-specific inducible nitric-oxide synthase expression is sufficient to cause hepatic insulin resistance and mild hyperglycemia in mice. *Journal of Biological Chemistry, 286*(40), 34959-34975.

Sinatra, S. (2004). *Anti-aginig Therapeutics Volume VI* Vol. 6. R. K. a. R. Goldman (Ed.) *"Fire in the Heart": New Developments in Diagnosis, Prevention & Treatment of Cardiovascular Disease* (pp. 27-45http://www.a24m.com/assets/pdf/bookstore/thera26_ch24.pdf?SESSION_MAIN=dap23mjgnb96luhbje28tcklgp75).

Sivasinprasasn, S., Sa-Nguanmoo, P., Pratchayasakul, W., Kumfu, S., Chattipakorn, S. C., & Chattipakorn, N. (2015). Obese-insulin resistance accelerates and aggravates cardiometabolic disorders and cardiac mitochondrial dysfunction in estrogen-deprived female rats. *Age (Dordr), 37*(2), 9766. doi: 10.1007/s11357-015-9766-0

Sobrino Crespo, C., Perianes Cachero, A., Puebla Jimenez, L., Barrios, V., & Arilla Ferreiro, E. (2014). Peptides and food intake. *Front Endocrinol (Lausanne), 5*, 58. doi: 10.3389/fendo.2014.00058

Song, Y., Manson, J. E., Tinker, L., Howard, B. V., Kuller, L. H., Nathan, L., . . . Liu, S. (2007). Insulin sensitivity and insulin secretion determined by homeostasis model assessment and risk of diabetes in a multiethnic cohort of women: the Women's Health Initiative Observational Study. *Diabetes Care, 30*(7), 1747-1752. doi: 10.2337/dc07-0358

Soni, A. C., Conroy, M. B., Mackey, R. H., & Kuller, L. H. (2011). Ghrelin, leptin, adiponectin, and insulin levels and concurrent and future weight change in overweight, postmenopausal women. *Menopause, 18*(3), 296-301.

Stanhope, K. L., Schwarz, J. M., Keim, N. L., Griffen, S. C., Bremer, A. A., Graham, J. L., . . . Havel, P. J. (2009). Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest, 119*(5), 1322-1334. doi: 10.1172/jci37385

Svendsen, O. L., Hassager, C., & Christiansen, C. (1995). Age- and menopause-associated variations in body composition and fat distribution in healthy women as measured by dual-energy X-ray absorptiometry. *Metabolism, 44*(3), 369-373.

Szanto, I., & Kahn, C. R. (2000). Selective interaction between leptin and insulin signaling pathways in a hepatic cell line. *Proc Natl Acad Sci U S A, 97*(5), 2355-2360. doi: 10.1073/pnas.050580497

Tan, B., Yin, Y., Liu, Z., Tang, W., Xu, H., Kong, X., . . . Wu, G. (2011). Dietary L-arginine supplementation differentially regulates expression of lipid-metabolic genes in porcine adipose tissue and skeletal muscle. *J Nutr Biochem, 22*(5), 441-445. doi: 10.1016/j.jnutbio.2010.03.012

Taylor, E. N., & Curhan, G. C. (2008). Fructose consumption and the risk of kidney stones. *Kidney Int, 73*(2), 207-212. doi: 10.1038/sj.ki.5002588

Thomas, S., & Schauer, P. (2010). Bariatric surgery and the gut hormone response. *Nutr Clin Pract, 25*(2), 175-182. doi: 10.1177/0884533610361739

Tolhurst, G., Zheng, Y., Parker, H. E., Habib, A. M., Reimann, F., & Gribble, F. M. (2011). Glutamine triggers and potentiates glucagon-like peptide-1 secretion by raising cytosolic Ca2+ and cAMP. *Endocrinology, 152*(2), 405-413. doi: 10.1210/en.2010-0956

Toth, M. J., Poehlman, E. T., Matthews, D. E., Tchernof, A., & MacCoss, M. J. (2001). Effects of estradiol and progesterone on body composition, protein synthesis, and lipoprotein lipase in rats. *Am J Physiol Endocrinol Metab, 280*(3), E496-501.

Toutouzas, K., Riga, M., Stefanadi, E., & Stefanadis, C. (2008). Asymmetric dimethylarginine (ADMA) and other endogenous nitric oxide synthase (NOS) inhibitors as an important cause of vascular insulin resistance. *Horm Metab Res, 40*(9), 655-659. doi: 10.1055/s-0028-1083814

Troke, R. C., Tan, T. M., & Bloom, S. R. (2014). The future role of gut hormones in the treatment of obesity. *Ther Adv Chronic Dis, 5*(1), 4-14. doi: 10.1177/2040622313506730

Tschop, M., Weyer, C., Tataranni, P. A., Devanarayan, V., Ravussin, E., & Heiman, M. L. (2001). Circulating ghrelin levels are decreased in human obesity. *Diabetes, 50*(4), 707-709.

Twito, O., Frankel, M., & Nabriski, D. (2015). Impact of glucose level on morbidity and mortality in elderly with diabetes and pre-diabetes. *World J Diabetes, 6*(2), 345-351. doi: 10.4239/wjd.v6.i2.345

Vilsboll, T., Krarup, T., Deacon, C. F., Madsbad, S., & Holst, J. J. (2001). Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes, 50*(3), 609-613.

Wallace, T. M., Levy, J. C., & Matthews, D. R. (2004). Use and abuse of HOMA modeling. *Diabetes Care, 27*(6), 1487-1495.

Wang, H., Wang, A. X., Aylor, K., & Barrett, E. J. (2013). Nitric oxide directly promotes vascular endothelial insulin transport. *Diabetes, 62*(12), 4030-4042. doi: 10.2337/db13-0627

Wettergren, A., Schjoldager, B., Mortensen, P. E., Myhre, J., Christiansen, J., & Holst, J. J. (1993). Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. *Dig Dis Sci, 38*(4), 665-673.

Wijnands, K. A., Castermans, T. M., Hommen, M. P., Meesters, D. M., & Poeze, M. (2015). Arginine and Citrulline and the Immune Response in Sepsis. *Nutrients, 7*(3), 1426-1463. doi: 10.3390/nu7031426

Wu, G., Collins, J. K., Perkins-Veazie, P., Siddiq, M., Dolan, K. D., Kelly, K. A., . . . Meininger, C. J. (2007). Dietary supplementation with watermelon pomace juice enhances arginine availability and ameliorates the metabolic syndrome in Zucker diabetic fatty rats. *J Nutr, 137*(12), 2680-2685.

Wu, G., & Morris, S. M., Jr. (1998). Arginine metabolism: nitric oxide and beyond. *Biochem J, 336 (Pt 1)*, 1-17.

Yoshizumi, M., Perrella, M. A., Burnett, J. C., Jr., & Lee, M. E. (1993). Tumor necrosis factor downregulates an endothelial nitric oxide synthase mRNA by shortening its half-life. *Circ Res, 73*(1), 205-209.

Zander, M., Madsbad, S., Madsen, J. L., & Holst, J. J. (2002). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet, 359*(9309), 824-830. doi: 10.1016/s0140-6736(02)07952-7

Vita

Thomas Jacob Jurrissen III was born in Summit New Jersey to Thomas and Brigitte Jurrissen. He graduated from Reidsville High School in 2008. The following autumn, he enrolled in Appalachian State University to pursue a Bachelor of Science degree in Exercise Science. In the fall of 2013, he accepted a graduate assistantship in the human performance lab, and later a research assistantship in Exercise Science and began study towards a Master of Science degree at Appalachian State University. The Master of Science degree was earned in May of 2015. In August 2015, Mr. Jurrissen will commence his work toward a Ph.D in Nutrition and Exercise Physiology at the University of Missouri.