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The purpose of this study was to determine if diet and/or body composition influences resting plasma apelin concentration. Apelin concentration appears to be influenced by the amount of fat in the body and by blood glucose concentration. However, most of the studies that have investigated blood apelin concentration have utilized obese or diabetic subjects. Little is known about how body composition, body fat distribution or diet may influence apelin in apparently healthy, young individuals. More specifically, the purposes of this study were to determine if baseline resting plasma apelin concentration in young, apparently healthy adult subjects is influenced by amount of fat in the body, location of fat, the macronutrient composition in their diet and if the total amount of antioxidant micronutrients (vitamins A, C, E and zinc). The proposed cohort group consisted of twelve apparently healthy young adults between the ages of 18-35.The amount of body fat, body mass index, sagittal diameter and the waist to hip ratio were utilized as covariates to determine if these factors influenced apelin concentration at rest. The data was analyzed to ascertain if there are any relationships with the listed anthropometric measures, the nutrition factors and plasma apelin concentration. Each subject's body composition was classified via body mass index (BMI), sagittal abdominal diameter (SAD), waist circumference (WC) and 7-site skinfold analysis. In addition, Each subject completed a three day diet record prior to their three visits which were analyzed for total calories, macronutrient percent calories and amount of micronutrient antioxidants; vitamin A, C, E, and zinc. They were instructed on how to log 3 day diet

records and were asked to repeat the diets as close as possible on their 2nd and 3rd visits to keep this information as consistent as possible. Significant relationships were found for body composition factors including Siri % body fat (r=0.631, P=0.028*), Brozek % body fat (r=0.642, P=0.024*), and BMI (r=0.649, P=0.022*). Body composition factors showed varied results. A significant association was found between sagittal abdominal diameter and plasma apelin concentrations at rest (r=0.628, p=0.029*), however waist circumference approached significance (r=0.061, P=0.061) and waist-to-hip ratio did not demonstrate a significant relationship (r=0.178, P=0.579). Only two subjects reported consuming high fat diets (>35% of total kilocalorie intake), therefore no relationships could be analyzed regarding plasma apelin concentration at rest and high fat diets. No significant relationships were found between individual micronutrients and plasma apelin concentrations at rest, besides vitamin E (r=0.658, P=0.020*). In conclusion this small cohort of subjects had stable resting plasma apelin levels across visits and does not appear that dietary factors influenced plasma apelin concentrations. In contrast, % BF and SAD suggest that these factors are significantly related to resting plasma apelin, with WC also approaching significance. More research should be done with a larger cohort of subjects including a wider range of diet and anthropometric measures.

ANALYZING THE ROLE OF BODY COMPOSITION AND DIET IN PLASMA APELIN LEVELS OF NORMAL HEALTHY ADULTS

by

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Approved by

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APPROVAL PAGE

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

Due to the exorbitant health care costs associated with the conditions and diseases associated with being classified as overweight or obese there has been much interest in obesity research. The National Health and Nutrition Examination Survey (NHANES) conducted by the Center for Disease Control and Prevention (CDC) provides longitudinal insight on the obesity trends in the United States via Body Mass Index (BMI) calculations collected in labs across the country (Flegal, 2012). The NHANES 2009-2010 data showed that the age-adjusted obesity (BMI $\geq$ 30) prevalence for both men and women was 35.7%, with no significant difference in prevalence between genders(Flegal, 2012). More alarmingly, the age-adjusted prevalence for overweight and obesity in both genders (BMI $\geq$ 25) was calculated to be 68.8% (Flegal, 2012). BMI is an important tool in assessing the weight status of populations, but on an individual basis, obesity is more accurately defined as greater than two standard deviations above the norm for percent body fat (%BF). The normal %BF for young men is 15 ± 5% and women is 21± 5%.Therefore, obesity would be considered greater than 25% body fat in young men and greater than 32% body fat in young women. On an international level, statistics show that about 1 billion people worldwide are overweight, with 300 million being obese (Bełtowski, 2006).

Table 1. BMI Normative Data. Source: US Dept. of Health and Human Services: NIH National Heart, Lung and Blood Institute
http://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmi_dis.htm

BMI Normative Data

Category	BMI (kg/m2)	Disease Risk Relative to Normal Weight & Waist Circumference	
Underweight	< 18.5	Men < 102 cm	Men > 102 cm
Normal	18.5-24.9	Women <88 cm	Women > 88 cm
Overweight	25.0-29.9	Increased	High
Obesity (I)	30.0-34.9	High	Very High
Obesity (II)	35.0-39.9	Very High	Very High
Extreme Obesity (III)	> 40.0	Extremely High	Extremely High

Table 2. Percent Body Fat Normative Data. Source: ACSM, 2006; IOM, 2002

Percent Body Fat Normative Data

Source:	**ACSM, 2006**		**IOM, 2002**		
Category (Percentile)	**Men (20-29)**	**Women (20-29)**	**Category**	**Men**	**Women**
Excellent (80-100)	5-10%	12-17%	Excellent	10-15%	20-24%
Good (60-79)	10.1-14%	17.1-21%	Good	16-20%	25-30%
Average (40-59)	14.1-17%	21.1-24%	Overweight	21-24%	31-36%
Low (20-39)	17.1-22%	24.1-28%	Obese	25-30%	37-41%
At Risk (0-19)	> 22%	> 28%	High Risk	> 31%	> 42%

The obesity epidemic is of particular importance due to increased prevalence of comorbidities and disease including but not limited to Type 2 Diabetes (T2D), cardiovascular disease (CVD), hypertension (HTN), hyperlipidemia and several types of cancers, as well as musculoskeletal, sleep and gallbladder problems (Dusserre, Moulin, & Vidal, 2000; Finkelstein, Fiebelkorn, & Wang, 2004).Obesity-related medical complications contribute substantially to the United States' $75 billion spent annually on medical expenditures, with an estimated 50% being funded by Medicare and Medicaid (Finkelstein et al., 2004). Estimates show that compared to normal weight individuals, obese patients incurred 36% greater medical expenses(Sturm, 2002).

It has been shown that obese patients are at increased risk for T2D, which is related to insulin resistance (IR)(N. P. E. Kadoglou, Vrabas, Kapelouzou, & Angelopoulou, 2012). Yue et al., defines insulin resistance as, "a diminution in a cell, tissue or organism's ability to take up glucose in response to insulin, [and] is the pathophysiological hallmark of type 2 diabetes mellitus" (Yue et al., 2010).Insulin resistance can be determined by the amount of insulin needed to handle a specific glucose load. Obesity-related IR occurs in a number of metabolic tissues including the liver, skeletal muscle, and adipose tissue (AT).

Obesity is linked to many variables, most commonly including sedentary behavior and overeating. The increased adiposity associated with high caloric consumption and imbalances in macronutrient intake has been shown to induce hypertrophic obesity, the increase in size of fat cells and or hyperplastic obesity, an increase in number of fat cells, or a combination of the two (Jo et al., 2009).

Nutrition, for example, total calorie consumption and the percentage breakdown of macronutrients, particularly the amount of fat in the diet can influence amount of AT stores. An overconsumption of macronutrients, including carbohydrates (CHO), protein, and/or lipids is termed nutrient overload and may contribute to metabolic dysfunction and ultimately IR. Similarly, an imbalance in consumption of the recommended ranges of macronutrients, along with the "nutrient overload" may lead to obesity along with increased risk for T2D(Alhazmi, Stojanovski, McEvoy, & Garg, 2012). Meta-analyses showed that high CHO and high fat diets (HFD) play a role in altering insulin resistance, and thus, risk for T2D (Alhazmi et al., 2012). High caloric intake of these nutrients leads to storage of triglycerides (TGs) in white adipose tissue (WAT), inducing adipose tissue expansion, and ultimately increased body fat, possibly inducing obesity. The overconsumption of CHOs and fats overload the body with calories, in which case the excess is stored as fat. The increased risk associated with high-CHO diets is due to the high glycemic index/load, which may lead to increased insulin demand, elevated plasma glucose concentrations, and ultimately contributing to pancreatic failure and glucose intolerance (T2D) (Alhazmi et al., 2012). It has also been reported that females consuming diets high in vegetable fats may see a decreased risk for T2D and increased insulin sensitivity through improvement in lipid profiles and better glycemic control in diabetics (Alhazmi et al., 2012).

Along with macronutrient consumption, intake of micronutrients also plays a significant role in proper physiological functioning of the body. Micronutrients are substances (vitamins and minerals), which are required, albeit in small amounts, for

proper development as well as prevention of disease and dysfunction (CDC, Linus-Pauling Institute).It is important that the diet consists of adequate amounts of micronutrient vitamin and minerals for proper physiological functioning; micronutrients are not synthesized by the body, therefore adequate consumption is required, as they must be derived from dietary intakes (CDC, LPI).

There are several noteworthy physiological complications associated with overweight and obese status. Insulin resistance, chronic inflammation, and oxidative stress are three possible physiologically significant consequences seen in overweight or obese humans. Due to WAT's plasticity, an increased amount of adipose tissue is a characteristic of obesity and may illicit metabolic changes (McArdle, Finucane, Connaughton, McMorrow, & Roche, 2013). These processes are thought to be regulated by various molecules secreted and released by adipocytes within the adipose tissue called "adipokines" or "adipocytokines." These molecules are thought to regulate many physiological processes associated with complications experienced due to overweight or obese states.

Recently, apelin, an adipokine, has been implicated in the regulation of obesity and increased risk of T2D. This recently discovered adipokine, is a signaling molecule which works on the APJ receptor, originates in adipose tissue, and may be affected by blood glucose levels and states of obesity as well as the amounts of vitamin C and insulin present(García-Díaz, Campión, Milagro, & Martínez, 2007; Langelaan, Bebbington, Reddy, & Rainey, 2009). It has been suggested that apelin expression is mediated by the severity of IR in diabetic subjects and by its location in various tissues (Dray et al.,

2010). A recent review discussed the etiology of IR as "diet-induced" via nutrient overload, through which cellular reduction is increased in the mitochondria, thus elevating the concentrations of hydrogen peroxide (H_2O_2) a potent radical inducer, creating a metabolic imbalance (Fisher-Wellman & Neufer, 2012).

Constant nutrient overload can influence reactive oxygen species (ROS) production and IR, inducing a state of chronic low-grade inflammation, oxidative stress, and possibly influencing apelin concentration(Garcia-Diaz et al., 2011). The expansion of AT due to increasing lipid droplet size has recently been implicated in the increased secretion and regulation of inflammatory cytokines, which was associated with the chronic state of low-grade inflammation and obesity along with IR-induced diabetes mellitus (Galic, Oakhill, & Steinberg, 2010).Thus, the amount of AT and potentially the site of adiposity can influence not only ROS, but also inflammation, and theoretically IR. Along with general obesity complications, it is commonly known that there is increased metabolic risk associated with carrying visceral (abdominal/central) adiposity as opposed to gynoid (peripheral/gluteophemoral) adiposity, likely due to increased free fatty acid circulation (Smith et al., 2001). Interestingly, studies have shown that lean subjects with an elevated waist-to-hip ratio (WHR), indicating central adiposity, have the highest risk for cardiovascular (CV) risk factors including IR, dyslipidemia, HTN, smoking, fibrinolysis, and various psychosocial factors (Björntorp, 1997). More recently, studies have taken into account sagittal abdominal diameter (SAD) as a correlate for elevated CV risk.

Table 3. Cutoffs for Determining Elevated Cardiovascular Risk. Lists the cutoffs for Sagittal Abdominal Diameter (SAD), Waist Girth (WG), Waist-Hip-Ratio (WHR), and Body Mass Index (BMI in kg/m^2).*Journal of Obesity* (2010).

Cutoffs for Determining Elevated Cardiovascular Risk

Measure	Men (95% CI)	Women (95% CI)
SAD (cm)	22.2 (21.6-22.8)	20.1 (19.4-20.8)
WG (cm)	100 (96.9-101)	88.4 (86.7-90)
WHR	0.97 (0.95-0.99)	0.82 (0.81-0.84)
BMI	27.9 (26.8-29)	27.6 (27-28.3)

The increase in AT associated with obesity can be deposited in various locations, but will mainly accumulate as WAT in various fat pads throughout the body. WAT influences the physiological function of lipid and glucose metabolism along with possessing endocrine function (M.-J. Lee, Wu, & Fried, 2013). Visceral adipose tissue (VAT) increases glucose uptake and metabolic activity in human subjects, *in vivo*, when compared to subcutaneous adipose tissue (ScAT)(Christen et al., 2010).

Therefore, the type of diet as well as macronutrients and micronutrients consumed are important factors that could influence plasma apelin concentration at rest. In addition, the amount of body fat and its location may also alter resting plasma apelin concentrations.

Statement of Problem

The purposes of this study are to determine if baseline plasma apelin concentrations in apparently healthy, young adult human subjects are influenced by body composition, body fat distribution, and diet.

Objectives

Currently, there are no known "normal" baseline plasma apelin levels for healthy populations. There has been research done on apelin levels in obese, diabetic, and several other special populations, but limited congruent data has been found in normal adult populations. This study examined the baseline plasma apelin levels in normal, apparently healthy adult human subjects, along with three-day food logs and anthropometric measurements. On the first visit, 7-site skinfold as well as SAD, BMI, and WHR were measured and used to determine the participants' body fat composition and distribution. Correlational analyses were conducted between %BF and plasma apelin concentration both at rest. Also, with the analysis of the subjects' diets, percentage of macronutrients and micronutrients were calculated in order to probe for relationships between consumption of specific macronutrients and micronutrients and apelin concentration. The antioxidant concentrations of several micronutrients were also determined to ascertain if an association between these antioxidants intake influenced apelin concentration.

Hypotheses

1. A positive relationship will exist between body fat percentage and plasm aapelin concentration at rest.
2. The location of fat (visceral) as determined by sagittal abdominal diameter (SAD) and waist-to-hip ration (WHR) will influence the plasma concentration of apelin at rest.

3. Individuals consuming a high fat diet should have a higher apelin concentration at rest.
4. Individuals with low antioxidant consumption will have a higher apelin concentration at rest than those with adequate antioxidant consumption in their diets.

Individuals with high zinc consumption will have a lower plasma apelin concentration than those with low zinc intake.

Limitations

- Diet for each subject was not controlled, but was indirectly monitored; each subject will be asked to maintain his or her normal diets throughout the study. They were given a3-day food log so they recorded their dietary intakes and were asked to reproduce their diets for each visit. Subjects may not have reported their food intakes correctly; therefore, it is possible that the food logs may not reflect the exact amount of the macronutrients and micronutrients ingested.
- Physical activity: Subjects were told to maintain their normal activities throughout the study. The pre-participation screening reflected their typical physical activity, but it is not the intention of this study to change their activity patterns.
- Sleep: Subjects were asked to maintain their normal sleep patterns throughout the study. It is not known if sleep patterns influenced apelin and associated processes.
- Alcohol consumption: Subjects were told not to ingest any alcohol at least 24 hours prior to any experimental day.

- Eating behaviors (vegetarian/paleo/portion sizes): It will be unknown whether the subjects actually consumed all of the nutrients provided in the food log as well as if they correctly reported portions sizes.
- Menstrual cycle was not assessed or monitored for the female subjects.
- Genetics may be a factor and was not controlled.
- The caloric balance of the subjects was not evaluated nor controlled.

Delimitations

- Apparently healthy subjects, aged 18-35, non-smokers and those not taking any medications or supplements known to affect fat and glucose metabolism were recruited.
- Subjects were required to keep a three-day dietary record prior to both exercise sessions and the glucose load to assess dietary factor influences on apelin.
- Subjects were required to discontinue any vitamin and antioxidant supplementation at least 2 weeks prior to any testing as this may influence markers of oxidative stress.
- Subjects were not on any medications that might influence catecholamines and/or glucose, insulin or fat metabolism.
- The subjects were non-smokers, not utilizing any tobacco products throughout the study.
- No subject reported being ill or sick during their testing.
- No subject reported any inflammation during their testing.

- Subjects reported to the laboratory in a post-absorptive state (at least 10 hours) and at the same time of day to avoid diurnal influences.
- All measures of %BF, Height, Weight, WHR, WC, and SAD were determined by a single experimenter to minimize variability and utilized the same landmarks on all subjects.
- All skinfolds were obtained by the same researcher, pinching the right side of the body at the various skin-fold sites at least 2 times.

Variables

Independent Variables:

- Diet
    - Total kilocalories consumed
    - Macronutrient content
    - Antioxidant amount
- Overweight/obese state
    - BMI (kg/m^2)
    - Sagittal Abdominal Diameter (SAD)
    - % Body fat (7 site skin-fold)
- Visceral vs. gynoid obesity
    - SAD
    - WC
- Dependents Variables:
- Apelin concentration at rest

CHAPTER II

REVIEW OF LITERATURE

Obesity and Body Fat Distribution

Obesity can be characterized by the well-known consequence of increased fat mass, or increased amounts of AT. AT is composed of fat cells, referred to as adipocytes and preadipocytes (adipocyte precursor cells) along with vasculature and nervous tissue (Avram, Avram, & James, 2005). AT is generally labeled as either brown adipose tissue (BAT) or WAT. Brown adipocytes are generally thought to be related to thermogenesis, via releasing cellular heat from food energy (Avram et al., 2005). WAT primarily functions to store excess lipid and responds to activators to mobilize the storage form of TGs in response to deprivation of food or enhanced energy need (exercise) (McArdle et al., 2013). WAT can be separated into intra-abdominal (VAT) and ScAT. Intra-abdominal fat is comprised of intraperitoneal and retroperitoneal fat, whereas ScAT can also be further divided into superficial and deep tissue layers (Avram et al., 2005). Intra-abdominal fat is mainly made up of intraperitoneal fat, which is further subdivided into omental (on stomach) or mesenteric (intestine) and epiploic (colon) fat tissue(Avram et al., 2005).

Due to WAT plasticity, adipocyte hypertrophy can expand the WAT during weight gain, which up-regulates the adipokines apelin and leptin as well as inflammatory cytokines (McArdle et al., 2013). There also may be differences in endocrine function

based on location of AT. VAT exhibits higher expression of plasminogen activator 1(PAI-1) than subcutaneous adipocytes, possibly leading to increased blood clots or thrombosis in patients with T2D and obesity (Dusserre et al., 2000). VAT also increases glucose uptake and metabolic activity in human subjects, *in vivo*, when compared to ScAT, possibly due to up-regulation of hexokinase (HK)-1 found in VAT-derived stromal vascular cells (SVCs) (Christen et al., 2010). A negative consequence associated with increased visceral adiposity is the over-expression of pro-inflammatory cytokines including: interleukin-6 (IL-6), interleukin 8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), Regulated on Activation, Normal T Expressed and Secreted (RANTES) recently renamed chemokine ligand 5 (CCL5), macrophage inflammatory protein-1α (MIP-1α), and PAI-1, whereas leptin and interferon gamma-induced protein 10 (IP-10) are expressed more in ScAT(M.-J. Lee et al., 2013).

Another negative consequence associated with obesity is oxidative stress, or a situation in which ROS accumulation occurs because the rate of production exceeds the rate of the antioxidant system's scavenging ability exacerbating metabolic dysfunction and microvascular complications associated with hyperglycemic-induced diabetes (Scott & King, 2004). It is important to look at the subject's antioxidant intakes due to their ROS scavenging properties and ability to attenuate oxidative stress and ultimately decrease the chronic inflammation associated with obesity and its comorbidities. ROS are also referred to as free radicals, a "species containing one or more unpaired electrons in their outer atomic orbital" (Nuttall, Kendall, & Martin, 1999). This electron imbalance renders free radicals highly reactive and capable of interacting with many substances

such as lipids, proteins, DNA and carbohydrates. The oxidation of these molecules can lead to disruption of protein structure, changes in cell membrane permeability, alteration of DNA transcription and influence carbohydrate storage, eventually causing a disruption of cell membranes leading to release of contents and cell death (apoptosis) (Nuttall et al., 1999; Scott & King, 2004).

Humans Studies:

It is well documented that visceral adiposity/central obesity is a strong correlate to obesity-related disorders including hypertriglyceridemia, glucose intolerance, HTN, and IR (Jensen, 1989). Increased expression of pro-inflammatory cytokines and IR-inducing factors lead to increased metabolic disturbances in humans with increased visceral adiposity, eliciting central obesity (Antuna-Puente, Feve, Fellahi, & Bastard, 2008; Dolinková et al., 2008; Fujioka, Matsuzawa, Tokunaga, & Tarui, 1987). While it is well known that apelin has a positive relationship with obesity, more specifically it has shown relationships with BMI (Chen et al., 2014; Heinonen et al., 2005; Boucher, 2005, Sheibani, 2012). However, to date, few if any studies have evaluated the relationship between visceral adiposity and plasma apelin. It has been reported that subjects with central obesity and increased VAT accumulation have increased apelin levels, although measured via different methodology and utilizing diseased populations (Klöting, 2008, Schinzarí, 2014). Several publications have reported WHR, WC and BMI as health risk, however these studies did not evaluate relationships between these factors and apelin concentration (Besse-Patin et al., 2014; Heinonen et al., 2009; N. P. E. Kadoglou et al.,

2012; N. P. Kadoglou et al., 2013; Sheibani, Hanachi, & Refahiat, 2012). Furthermore, we are not aware of any report that has compared SAD with apelin.

Animal Studies:

Due to the novelty of the apelinergic system, much of the current research has utilized animals, rather than human controls. In 2005, Boucher et al. demonstrated that the apelin gene (APLN) is expressed by both intra-abdominal and ScAT in mice (Boucher et al., 2005). A more recent study reported that expression of APLN was higher in retroperitoneal WAT compared to subcutaneous WAT in rats (García-Díaz et al., 2007). Garcia-Diaz et al. also reported that following 56 days of high-fat diet (HFD) feeding, rats demonstrated a higher expression of subcutaneous, but not retroperitoneal APLN gene expression(García-Díaz et al., 2007). Adipogenesis can be inhibited by apelin/APJ via lymphatic and blood vessel integrity blocking the growth of lipid droplets in HFD-fed rats (Sawane, Mika Kajiya, Kentaro Kidoya, Hiroyasu Takagi, Masaya Muramatsu, Fumitaka Takakura, 2014).

Diet: Macronutrients

It is important to examine diet when investigating apelin due to the potential influence macronutrient and micronutrient intake may have on metabolism and adipokine expression. The three main macronutrients considered building blocks of energy for the body's physiological functioning are CHO, proteins and lipids. The United States Department of Health and Human Services (HHS) and the U.S. Department of Agriculture (USDA) have published joint recommendations for daily energy intakes, stating that the diet should consist of 45-65% carbohydrates, 10-35% protein, and 20-

35% fat. This recommendation is based on their proposed 1,800-2,400 kilocalorie per day consumption by females and 2,400-3,000 kilocalorie per day allowance for males (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010).

Table 4. Recommended Macronutrient Intakes (%). Source: U.S. Department of Agriculture, U.S. Department of Health and Human Services; www.dietaryguidelines.gov 2010

Recommended Macronutrient Intakes (%)			
	Carbohydrate (%)	**Protein (%)**	**Fat (%)**
Adults (19 years and older)	45-65%	10-35%	20-35%

Table 5. Estimated Caloric Needs Per Day. Source: U.S. Department of Agriculture, U.S. Department of Health and Human Services; www.dietaryguidelines.gov 2010

Estimated Caloric Needs Per Day			
Gender	**Sedentary**	**Moderately Active**	**Active**
Males (19-30)	2,400-2,600	2,600-2,800	3,000
Female (19-30)	1,800-2,000	2,000-2,200	2,400

Table 6. Percentages of Energy from Protein, Carbohydrates and Fat by Gender and Age in the US. Source: USDA: NHANES "What We Eat in America" 2011-2012

Percentages of Energy from Protein, Carbohydrates and Fat by Gender and Age in the US

Gender	Energy (kcal)	Protein (% kcal)	CHO (% kcal)	Total Fat (% kcal)	SFAs (% kcal)	MUFAs (% kcal)	PUFAs (% kcal)
Males (20-29)	2764 (74.5)	16 (0.3)	48 (0.8)	33 (0.7)	11 (0.3)	12 (0.4)	8 (0.3)
Females (20-29)	2019 (41.6)	15 (0.2)	51 (0.6)	33 (0.4)	11 (0.2)	12 (0.2)	8 (0.2)
Males and Females (20 and over)	2191 (15.6)	16 (0.1)	49 (0.3)	33 (0.2)	11 (0.1)	12 (0.1)	8 (0.1)

Similarly, the Institute of Medicine (IOM) has published Recommended Dietary Allowances (RDAs) and Acceptable Macronutrient Distribution Ranges (AMDRs) for macronutrients. RDAs are recommended allotments of each macronutrient in grams per day representing the amount needed to meet the needs of almost all (97-98%) individuals. AMDR varies from RDA, representing an acceptable range of macronutrient intake associated with decreasing the risk of chronic disease and providing adequate intakes of essential nutrients (Institute of Medicine, 2005). The RDA for carbohydrates is 130 g/day, whereas the AMDR is 40-65 g/day. The AMDR for total fat is 20-35 g/day, however, no RDA is provided due to the increased risk for subjects on a high fat diet(Institute of Medicine, 2005). These recommendations for fat provide adequate intake for the use in structural membrane lipids, cell signaling, skin function and use as a precursor of eicosanoids (Institute of Medicine, 2005). It is known that there is a relationship with saturated fats and increased risk for CVD(Alhazmi et al., 2012; Meza-

Miranda et al., 2014; Peairs, Rankin, & Lee, 2011). There are also concerns that trans-fats increase low density lipoproteins(LDLs) and decrease high density lipoproteins(HDLs), increasing the risk of coronary heart disease (CHD) as reported by the CDC (Ogden, Carroll, Kit, & Flegal, 2014). In association with CHOs and fats, it is important to maintain adequate protein intakes to build and remodel amino acids (AAs), function in enzymatic activity, and transport molecules and hormones. In order to prevent deficiency syndromes, the AMDR recommendation for protein is 10-35 g/day and the RDA is 56 g/day for males and 46 g/day for females (Institute of Medicine, 2005).

Table 7. Nutritional Goals for Age-Gender Groups, Based on DRIs and Dietary Guidelines. This table provides recommendations for macronutrients (USDA&DHHS, 2010). AI = Adequate Intake, RDA=Recommended Dietary Allowances, AMDR = Acceptable Macronutrient Distribution Ranges

Nutritional Goals for Age-Gender Groups, Based on DRIs and Dietary Guidelines

Nutrients (units)	Source of Goal	Female (19-30)	Male (19-30)
Protein (g)	RDA	46	56
Protein (% kcal)	AMDR	10-35%	10-35%
Carbohydrate (g)	RDA	130	130
Carbohydrate (% kcal)	AMDR	45-65 %	45-65%

Table 8. Macronutrient Intakes of Typical Americans**.** Source: USDA: NHANES "What We Eat in America" 2011-2012

Macronutrient Intakes of Typical Americans

Gender	Energy (kcal)	Protein (g)	CHO (g)	Total Fat (g)
Males (20-29)	2764 (74.5)	102.9 (3.08)	332 (11.1)	102.3 (2.63)
Females (20-29)	2019 (41.6)	72.1 (1.38)	255 (6.4)	75.5 (1.84)
Males and Females (20 and over)	2191 (15.6)	83.0 (0.75)	266 (2.4)	82.2 (0.77)

Human Studies:

These macronutrients exist in a delicate balance to supply the body with adequate energy supply during rest and work; imbalances, however may increase the risk for T2D (Alhazmi et al., 2012). Meta-analyses done by Alhazmi and colleagues (2012) show that high CHO and high vegetable fat diets play a role in altering IR and thus, risk for T2D. Macronutrient intake, particularly high-CHO consumption has been correlated with an increase in risk for T2D, whereas diets high in vegetable fat show a decrease in risk for T2D in women. Alhazmi's group reported that the increased risk associated with high-CHO diets was due to the high glycemic index/load, which may lead to increased insulin demand, elevated plasma glucose concentrations, and ultimately contributing to pancreatic failure and glucose intolerance, the pathophysiological hallmarks for T2D. It has also been reported that females consuming diets high in vegetable fats may see a decreased risk for T2D and increased insulin sensitivity through improvement in lipid profiles and better glycemic control in diabetics (Alhazmi et al., 2012).

Animal Studies:

Mei et al. demonstrated that a HFD (>37% fat in the diet) increased lipogenesis and elicited IR, and adding 5% CHO to this HFD significantly worsened HFD-induced oxidative stress, thus exacerbating IR and ectopic fat accumulation in mice. The addition of 10% CHO to this HFD induced maximal IR. This study suggests that CHOs are not necessary for HFD-induced IR to occur, possibly due to hepatic gluconeogenesis, but addition of CHOs to a HFD will dramatically aggravate IR induced by a HFD (Mei et al., 2014). In lean Zucker rats, postprandial oxidative stress was exhibited after eating a meal, while overweight and obese individuals experienced a constant state of low-grade inflammation and oxidative stress.

Diet: Micronutrients

While macronutrient intake is an important mediating factor in postprandial oxidative stress, micronutrient, specifically, antioxidant intake may play a substantial role in redox reactions and oxidative stress. It is suggested by the Food and Nutrition Board (FNB) of the IOM that the RDA for vitamin C is 90 mg/day for males and 75 mg/day for women, 900 mcg/day for men and 700 mcg/day for women in terms of vitamin A, and 15 mg/day for both genders for vitamin E. The RDAs listed for vitamin A are based on the amount of intake necessary to ensure adequate storage to support normal physiological functions. Both vitamin C and vitamin E are known antioxidants which can help reduce oxidative stress(Institute of Medicine, 2005).

Table 9. Nutritional Goals for Age-Gender Groups Based on DRIs and Dietary Guidelines. This table provides recommendations for micronutrients. Source: U.S. Department of Agriculture, U.S. Department of Health and Human Services; www.dietaryguidelines.gov 2010

Nutritional Goals for Age-Gender Groups, Based on DRIs and Dietary Guidelines

Micronutrient (units)	Source of Goal	Female (19-30)	Male (19-30)
Zinc (mg)	RDA	8	11
Vitamin A (mcg RAE)	RDA	700	900
Vitamin E (mg AT)	RDA	15	15
Vitamin C (mg)	RDA	75	90

Table 10. Typical Micronutrient Intakes of Americans. Source: USDA: NHANES "What We Eat in America" 2011-2012

Typical Micronutrient Intakes of Americans

Gender	Retinol (ug)	Vitamin A (RAE) (ug)	Beta-Carotene (ug)	Vitamin C (mg)	Vitamin E (alpha-tocopherol) (mg)	Zinc (mg)
Males (20-29)	458 (41.5)	612 (52.4)	1678 (191.4)	98.3 (10.72)	10.2 (0.35)	14.0 (0.38)
Females (20-29)	389 (22.8)	593 (36.3)	2192 (254.1)	83.0 (6.71)	8.0 (0.38)	10.0 (0.26)
Males and Females (>20)	444 (24.6)	674 (38.0)	2500 (181.7)	84.8 (4.10)	9.0 (0.16)	11.5 (0.15)

The main source of ascorbic acid (vitamin C) and carotenoids (vitamin A) are through dietary fruit and vegetable consumption, which are also a source of tocopherols (vitamin E). Micronutrient deficiency is an important issue because nationwide only

about 9% of Americans eat the recommended daily servings of fruits (5 servings) and vegetables (4 servings); similarly, the NHANES reports the typical American diet is deficient in vitamin E intake (Ames, Shigenaga, & Hagen, 2014). Vitamin E (tocopherols) can be found in nuts, vegetables, fruits, oil, and seeds; vitamin C (ascorbic acid) can be found in most citrus fruits and various vegetables; carotenoids are also found in fruits, vegetables and seaweeds (Osawa & Kato, 2005).

Antioxidants can be classified into three categories: metal ion binding proteins, intracellular antioxidant enzymes, and extracellular antioxidants. Certain antioxidants are considered metal binding and decrease the hydroxyl radical production such as transferrin, ceruloplasmin and albumin. There are also intracellular and extracellular antioxidant enzymes. Intracellular antioxidants include superoxide dismutase, catalase and selenium-dependent glutathione peroxidase, which are implicated in trapping free-radical by-products of cellular metabolism as enzymatic regulators. In addition, glutathione is an important non-enzymatic intracellular antioxidant, which helps donate a hydrogen ion to help reduce ROS. Antioxidants such as α-tocopherol, β-carotene, and vitamin C are considered non-enzymatic substances and help prevent or quench some of the ROS to lesser active molecules. In conjunction with the enzymes, a hydrogen donor is needed, often obtained from the molecule glutathione (GSH) and is returned to the oxidized form, glutathione disulfide (GSSG) with a NADPH donor via co-enzyme glutathione reductase. Thus, a major intracellular regulator of oxidative stress is the glutathione (thiol) reaction, which is also related to the reestablishment of both vitamin C

and E to their reduced forms (Ames et al., 2014; Antoniades, Tousoulis, Tentolouris, Toutouzas, & Stefanadis, 2003)

Human Studies:

In a recent study, it was suggested that micronutrient deficiencies have an effect on angiogenesis and chronic inflammation, also showing correlations to increased obesity rates among impoverished communities (Olga P García, Long, & Rosado, 2009). Low levels of vitamin C and vitamin E:lipids ratio have been correlated with obesity and overall low-grade systemic inflammation (Olga Patricia García et al., 2013). Observational studies suggest that vitamin A and/or C may be able to restore endothelial function and possibly have anti-inflammatory and anti-thrombotic properties; although, randomized control trials (RCTs) have not confirmed their role in inhibiting atherosclerosis (Antoniades et al., 2003). Obese and overweight children have lower concentrations of vitamin A and elevated triglycerides along with increases in C-reactive protein (CrP), whereas low vitamin E concentrations were associated with decreased glucose and triglycerides, along with higher LDLs (Olga Patricia García et al., 2013). Garcia et al. (2013) also reported that zinc deficiency was correlated with increased insulin resistance(Olga Patricia García et al., 2013). In 2007, Garcia-Diaz et al. showed that apelin gene expression was maintained at elevated levels even when vitamin C supplementation resulted in a decrease in adiposity and other obesity markers (García-Díaz et al., 2007). Therefore, more work is needed to understand the influence of vitamin C on apelin concentration.

Table 11. Percentage of Americans with Inadequate Dietary Intakes Based on EARs. Source: What We Eat in America, NHANES 2001-2002

Percentage of Americans with Inadequate Dietary Intakes Based on EARs	
Nutrient	**Percent**
Vitamin A	44%
Vitamin C	31%
Vitamin E	93%
Zinc	12%

A recent review by Garcia et al. (2009) reported that vitamin A deficiencies have been linked to elevated weight, BMI, and hip circumferences in overweight and obese subjects(Olga P García et al., 2009). Plasma retinol levels have been used as an indicator of vitamin A deficiencies and have been negatively correlated with overweight and obese children, as well as increased insulin resistance in morbidly obese adults (Olga P García et al., 2009). Dietary deficits of vitamin A intake have also been implicated in the increased incidence of obesity among humans, whereas retinoic acid (RA) supplementation was shown to decrease body weight, body fat, retroperitoneal WAT, and adipocyte size in mice(Olga P García et al., 2009). Similarly, Vaughan et al. (2007) reported a significant correlation between vitamin A deficiency and an increased incidence of obesity in Native American adults (Vaughan, Benyshek, & Martin, 1997). Several pathways have been proposed in terms of how RA might inhibit adipogenesis. Menendez, et al. suggested that RA and vitamin D_3 may exhibit a direct inhibitory effect on the secretion of leptin and pro-inflammatory cytokines from adipose tissue *in vivo*, which may also play an important role in regulation of food intake, energy expenditure

and body composition (Menendez et al., 2001). Another possible link between obesity and insulin resistance may be through retinol-binding protein (RBP4), a cytokine shown to be secreted from AT and the liver, and has been associated with increased BMI, IR, dyslipidemia, HTN, and visceral adiposity. It was proposed that RBP4 is up-regulated in response to decreased expression of glucose transporter (GLUT 4) within AT, which was suggested to lead to IR (D. F. Garcia-Diaz, J. Campion, F. I. Milagro, L. Paternain, 2009).

Table 12. Typical American Diets vs. Recommended Intake Levels. Source: U.S. Department of Agriculture, U.S. Department of Health and Human Services; www.dietaryguidelines.gov 2010

Typical American Diets vs. Recommended Intake Levels

Food Type	Usual intake as % of goal or limit
Whole Grains	15%
Vegetables	59%
Fruits	42%
Oils	61%
Fiber	40%
Calories from Solid fats/added sugars	280%
Refined Grains	200%
Sodium	149%
Saturated Fat	110%

Vitamin C is considered a potent water soluble, natural antioxidant in terms of its ROS scavenging properties within the cytoplasm and its NF-κB-mediated anti-inflammatory properties, as well as its role in regeneration of oxidized vitamin E to reduced vitamin E and conservation of β-carotene during times of oxidative stress

(Antoniades et al., 2003; Garcia-Diaz et al., 2011; Pedraza, Bo, & Golde, 2002). Vitamin C has been associated with a down-regulation of proteins implicated in several metabolic pathways: the pentose phosphate cycle, tricarboxilic-acid cycle, and progesterone and isoprenoid biosynthesis. Vitamin C has been shown to influence stimulation of adipogenesis, suggesting a link with these pathways and vitamin C (Campión, Milagro, Fernández, & Martínez, 2006). Campion and colleagues (2006) reported that vitamin C supplementation (75 mg/kg rat) in conjunction with a high fat diet decreased body weight and fat content (without affecting food intake) in rats, compared to a similar high fat, hypercaloric Western diet, without vitamin C. Thus, vitamin C demonstrated an anti-obesity protective property in rats, however this needs to be substantiated in humans (Campión et al., 2006). Pedraza and colleagues (2002) incubated cells with dehydroascorbic acid (DHA), the oxidized form of vitamin C, which may inhibit the TNF-α mediated activation of NF-κB- inducing kinase (NIK) and IkB (IKKβ) independent of the p38 MAP kinase pathway, influencing the inflammatory, neoplastic, and apoptotic cell processes via tumor necrosis factor-α(TNF-α) mediated NF-κB inhibition (Pedraza et al., 2002). Vitamin C treatment was associated with decreased body weight gain, and lower plasma leptin levels along with down-regulation of apelin gene expression in adipose tissue of rats (García-Díaz et al., 2007).

Vitamin E is partially regulated by vitamin C-mediated reduction of the oxidized form of vitamin E (the vitamin E radical), creating a synergistic effect between vitamin C and vitamin E (Nuttall et al., 1999). In terms of vitamin E, its primary component, α-tocopherol, is implicated in the antioxidant protective properties of oxidative

modification of lipids such as low-density lipoproteins (LDLs), whereas γ-tocopherol is associated with scavenging peroxynitrite-derived free radicals in the natural diet (Antoniades et al., 2003; Opie, 1997). Vitamin E is considered "the major lipid-soluble antioxidant" preventing the development of lipid hydroperoxides from PUFAs, and is found within LDL and all membranes, preventing lipid peroxidation (Antoniades et al., 2003).Although it is difficult to demonstrate vitamin E deficiency, since it is stored within lipids, dietary research has noted that vitamin E consumption is inadequate in most American diets(U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010). Therefore, it would be informative to understand if vitamin E and vitamin C consumption may influence apelin concentration and its response to stressors that theoretically should increase its release.

Olga et al. (2009) reported that low zinc intake and plasma zinc concentrations correlated with an increased incidence of obesity, specifically central adiposity and its associated comorbidities including heart disease and T2D. A possible mechanism by which a marginal zinc deficiency may alter adipogenesis is through a reduction in leptin concentration, possibly leading to increased risk for obesity (García-Díaz et al., 2007). In obese and overweight individuals, insufficient zinc intake increased oxidative stress and inflammatory responses associated with decreased superoxide dismutase activity and glutathione peroxidase, leading to reduced lean body mass and increased body fat (Olga P García et al., 2009). Therefore, adequate zinc intake within the diet may help attenuate some of the associated declines in enzymatic oxidative stress control and thus may reduce obesity.

Animal Studies:

Apelin has been implicated in the ROS scavenging of H_2O_2, considered a primary cause of insulin resistance in which nitric oxide (NOS)-stimulated hypothalamic hyperglycemia increases mitochondrial ROS (mROS) in the hypothalamus, thus initiating peripheral hyperinsulinemia, and increased glucose storage (Drougard et al., 2014; Fisher-Wellman & Neufer, 2012). Trolox (water-soluble vitamin E analog) has been shown to completely inhibit several processes including: apelin-induced hyperglycemia, increased H_2O_2 release from the hypothalamus, as well as the stimulation of hepatic glycogenolysis in apelin-injected mice (Drougard et al., 2014). Apelin has also been shown to activate glucose 6-phosphatase (G6Pase) enzymatic pathway, which implicates apelin's role in stimulation of glycogenolysis and gluconeogenesis in the liver of apelin-treated mice (Drougard et al., 2014).Furthermore, they also reported that Trolox and F13A (APJ antagonist) inhibited this apelin-induced stimulation of glycogenolysis and gluconeogenesis within the liver of mice, as well as completely blocked apelin-induced hyperglycemia in HFD mice (Drougard et al., 2014).

Garcia-Diaz et al. (2011) showed that the antioxidant vitamin C decreased macrophage induced inflammation via inhibition of NO and down-regulation of MCP-1 and apelin in mice preadipocytes. These results suggested that vitamin C may provide a pathway to improve insulin resistance not only through its control of oxidative stress and inflammation but also through apelin(Garcia-Diaz et al., 2011). Additional information regarding micronutrient influence on apelin regulation is limited. This emphasizes the

importance of analyzing diets and antioxidant consumption when analyzing baseline plasma apelin levels of normal, apparently healthy human populations.

Apelin

Apelin, a recently discovered 36 amino acid peptide, located on the Xq25-26.1 chromosome, is a cognate ligand for the G-protein coupled receptor (GCPR) referred to as APJ (Beltowski, 2006; Tatemoto et al., 1998). Tatemoto et al. (1998), first isolated apelin using bovine stomach extracts in 1998. Apelin's precursor, referred to as preproapelin, is comprised of a 77 amino acid sequence, from which several mature forms of the apelin peptide emerge, encoded by the gene APLN. The most abundant form of apelin, apelin-36, is thought to be a "mature form" of the apelin peptide, with apelin-12, apelin-17, apelin-13, and [pyr]-13 apelin to be less abundant but more potent (O'Carroll, Lolait, Harris, & Pope, 2013).

Human Studies:

Although research has expanded in regards to apelin, many of the studies are being done in rodents rather than human subjects. In human studies, much of the literature is conflicting, thus, it is important that apelin concentrations in normal, healthy, young controls are established (see Table 13). Researchers have shown apelin gene expression in the brain and peripheral tissues in the heart, spleen and liver of humans. Preproapelin was discovered in various regions of the brain including the caudate nucleus, thalamus, hypothalamus, hippocampus, midbrain, basal forebrain and frontal cortex (D. K. Lee et al., 2000).

Table 13. Previous Studies Reporting Plasma Apelin Concentrations in Healthy Human Subjects.

Previous Studies Reporting Plasma Apelin Concentrations in Healthy Human Subjects

Study	*Year*	*Apelin Concentration (ng/ml) ± SE*	*Kit*	*Origin*	*Procedure/Kit Utilized*
Zhen et al.	2013	0.1292 ± 0.0203	EIA	Burlingame, CA	Phoenix Pharmaceuticals: Apelin-12 HRMB
Mesmin et al.	2010	0.369 ± 0.08	EIA	Strasbourg, France	Phoenix Pharmaceuticals: Apelin-12 HRMB
Baso	2007	0.738±0.186	EIA	Belmont, CA	Phoenix Pharmaceuticals: Apelin-12 HRMB
Castan-Laurell et al.	2008	0.272 ± 0.02	EIA	Burlingame, CA	Phoenix Pharmaceuticals: Apelin-12 HRMB
Soriguer	2009	1.12±0.51	EIA	Belmont, CA	Phoenix Pharmaceuticals: Serum Apelin-12
Heinonen	2009	0.174±0.014	EIA	Belmont, CA	Phoenix Pharmaceuticals: Apelin-12 HRMB
Alexiadou et al.	2012	1.89 ± 0.14	EIA	CA	Phoenix Pharmaceuticals: Apelin-12 HRMB
Kadoglou et al.	2012	0.213 ± 0.114	EIA	Belmont, CA	Phoenix Pharmaceuticals: Apelin-12 HRMB
Ba et al.	2013	1.13±0.55	EIA	Belmont, CA	Phoenix Pharmaceuticals: Serum Apelin-12
Papadopoulos et al.	2013	0.315 ± 0.147	RIA	Burlingame, CA	Phoenix Pharmaceuticals: Apelin-12 HRMB

Animal Studies:

Similar to humans, rats express preproapelin in the frontal cortex, cortex striatum, midbrain, hippocampus, medulla, pons, and cerebellum of the brain, along with several peripheral tissues including pituitary, olfactory tubercle, septum, adrenal, vas deferens, testis, intestines and kidneys, as well as in rat fetuses (D. K. Lee et al., 2000).

Apelin, primarily secreted from adipocytes, is considered a novel adipokine, recently discovered, with little knowledge of its pathophysiological implications (Daviaud et al., 2006). Adipocytes, or fat cells have been shown to produce both apelin and the APJ (Langelaan et al., 2009). AT possesses endocrine function and has been implicated in communicating to other cells to regulate several factors including substances called adipokines, also referred to as adipocytokines(Schutte et al., 2010).

Apelin and Exercise

Kadoglou et al. (2012) suggested that along with other adipokines including visfatin and adiponectin, apelin is increased with physical activity lifestyle modifications among patients diagnosed with T2D. However, the influence in healthy young individuals has not yet been adequately determined.

APJ

APJ, gene symbol APLNR is known as a GCPR which sense extracellular molecules and act to achieve cellular responses via signal transduction. APJ acts as the receptor for the cognate ligand, apelin, and thus far has been found in the brain and peripheral tissues in both rodents and humans.APJ immunoreactivity staining showed less apelin expression in cardiomyocytes, glial cells of the brain, and vascular smooth

muscle cells in humans (Bełtowski, 2006; Langelaan et al., 2009). Research suggests that APJ expression is mediated by the severity of IR. Rodent studies showed decreased APJ expression in insulin-resistant mouse skeletal muscle and that APJ expression was not affected by acute feeding/refeeding (Castan-Laurell, Vítkova, Daviaud, Dray, Kováčiková, et al., 2008; Dray et al., 2008). However, there may be differences in APJ expression and IR for the various tissues in rodents and humans (Dray et al., 2010) Increased expression of the APJ gene occurs during times of acute and repeated stress, which was suggested to be related to glucocorticoid dependent mechanisms (O'Carroll et al., 2013). This is important in terms of obesity due to the chronic state of low-grade inflammation and oxidative stress exhibited with obesity and its comorbidities. APJ shows significant similarities to the angiotensin receptor, angiotensin II type 1 (AT1), ~30% homology to the angiotensin receptor, although the APJ receptor did not show affinity for angiotensin II (D. K. Lee et al., 2000).

To date, only one metabolic pathway has been found for apelin involving the zinc-containing carboxypeptidase, angiotensin-converting enzyme-2 (ACE2), which is involved in the conversion of angiotensin I to II into active forms found primarily in the endothelial vasculature of the heart, kidney and testis (Bełtowski, 2006). This is important in terms of the antioxidant role in apelin expression because the only known pathway involves ACE2, which is zinc-dependent. Therefore, we determined if the zinc consumption in our subjects was within the normal range as an index of its role in controlling enzymatic function related to oxidative stress and apelin expression. Additionally, we ascertained if there is a relationship between zinc consumption, total

antioxidant intake, total kilocalories consumed, percentages of macronutrients, body fat distribution, and body composition in relation to apelin at rest and in response to exercise and glucose-induced stress.

CHAPTER III

METHODS

Subjects

Subjects for this study were recruited on a volunteer basis. During the first visit the subjects reviewed and signed a written consent form according to University of North Carolina at Greensboro IRB standards as well as underwent a pre-participation screening (Appendix A/B). Fifteen subjects were recruited, however this data is part of a larger study, and therefore three subjects (2 males, 1 female) were not included due to abnormal glucose responses during the 54g glucose challenge. The reported values include twelve (n=8 M, n=7 F) apparently healthy, non-obese (skinfold & BMI) subjects between the ages of 18-35 and not using tobacco products or taking any medications or supplements that may alter antioxidant concentration, metabolism, blood glucose and/or insulin were recruited. Both male and female subjects were recruited in order to get a general baseline plasma apelin level in young, apparently healthy individuals.

Study Design

Inclusion/Exclusion Criteria:

Prior to acceptance into the study, potential participants attended a screening and consenting session in which potential medical, cardiovascular, physical activity and other possible confounders that may exist were addressed (Appendix A/B). Those excluded from the study included tobacco users of all forms and pregnant women as well as obese

individuals (BMI and %BF via skinfold analysis> 2 SD above norm for age category) and individuals who were not otherwise apparently healthy. Potential subjects were screened using American College of Sports Medicine (ACSM) CV risk factor assessment (ACSM Pre-Participation Health Screening – Appendix A); those meeting ≥ 2 CV risk factors were excluded from the study. Other exclusion criteria included those on medications and supplements known to influence metabolism, oxidative stress, and/or inflammation to prevent confounding outcome measures (Adapted Health History Questionnaire - Appendix B). Those included in the study were within the specified age range and meet all inclusion criteria to achieve a healthy population.

Screening/Consenting Session (Visit 1)

Before beginning the study, subjects attended a screening for health history and physical activity for use as part of inclusion and exclusion criteria (Appendix A/B). If they met all inclusion criteria and no exclusion criteria, they were asked to participate as a subject and read and sign the institutional review board (IRB) approved informed consent form. At this time, they were given ample time to ask questions and were informed of potential risks and benefits. Once the subject signed the informed consent form and completed screening, they were considered "active subjects." At this time the subjects' resting measures were recorded including heart rate, blood pressure, height and weight. These measures were used as inclusion/exclusion criteria, excluding subjects who did not exhibit normotensive blood pressure and those considered obese via BMI (kg/m^2). During this visit, the subject was also informed about their involvement and provided a study timeline, detailing their requirements, screening/consenting and all

interventions and their responsibilities. The subjects were given a three-day food log, instructed on how to properly input their diets, in which they recorded the specific food they consumed, amount of food, time consumed, how the food was prepared and caloric content if known. If subject had food labels, the subject was asked to submit the labels to the researcher for analysis. The macronutrient and micronutrient content was analyzed using USDA Food Nutrient Database, http://fnic.nal.usda.gov/food-composition/usda-nutrient-data-laboratory. The subjects were provided copies of their first 3-day food log and upon each visit, the participant was told to reproduce their originally recorded diets as consistently as possible for the consecutive visits. Upon receipt of each food log, the subject was prompted to ensure all cooking methods and possible added sources of nutrients were properly understood. Notes were made in regards to their measurement techniques as well as any condiments or cooking methods they may use. The macronutrients were converted to kilocalories using the standard conversions of 4 kcal per gram of CHO and Protein and 9 kcal per gram of fat (*Dietary Reference Intakes: Macronutrients*, 2005) . The average was taken from each visit and then compared across visits. There were no significant differences between visits; therefore the data was collapsed across visits, to provide an average kCal intake per subject for each macronutrient. The same was done for vitamin A, vitamin C, vitamin E, and %RDA TAC.

Subject Testing

The subjects were asked to arrive at the laboratory in the morning fasted in a post-absorptive state (6-10 hours) without consuming caffeine, alcohol or any contraindicated substance. The subjects arrived rested, not having exercised within the past 24 hours prior to the visit.

During the first visit, the subject was weighed (Seca scale) to the nearest .1 kg and height was measured using a stadiometer to the nearest 0.1 cm to determine BMI (kg/m^2). The equation for calculating BMI is as follows: BMI (kg/m^2) = body mass (kg)/height (m^2). To determine height, the subject was asked to remove their shoes, stand straight up with heels together, take a deep breath and hold it while looking straight ahead with their head level(National Heart Lung and Blood Institute & National Institutes of Health (NIH) National Heart, Lung, and Blood Institute, 1998). Heart rate was attained via brachial artery and blood pressure using an auscultatory cuff, measured on the right side of the body to assure participants remained within the inclusion criteria as well as to establish baseline measures. % BF was calculated using 7-site skinfold (chest, tricep, axilla, subscapular, abdominal, suprailium, thigh) (See Appendix G for location and direction of each skin fold site) using a Harpenden caliper that provides a constant pressure of ~10g/mm2. Each fold was measured to the nearest 0.5 mm on the right side of the body, with each measurement within 2 mm or a third reading was taken until at least two measures were within 2 mm of each other to ensure reliability and validity. The skinfold was grasped firmly between the thumb and index finger of the left hand, facing up the jaws of the caliper were placed 1 cm below the pinch (Ehrman, 2010; Maud &Foster,

2006).The average of each skinfold site was summed and put into the Jackson and Pollock body density equation, based on the fact that the majority of subjects were Caucasian(Jackson & Pollock, 1978). Using these equations, the subjects' lean body mass was also calculated and used in correlational analysis. The subjects' body density was then used to calculate % body fat using both the Siri and Brozek equations.

Body Density:

Males: $(\sum 7) = 1.112 - (0.00043499 \times \sum 7) + (0.00000055 \times (\sum 7)^2) - (0.00028826 \times \text{age})$

Females: $Db\ (\sum 7) = 1.097 - (0.00046971 \times \sum 7) + (0.00000056 \times (\sum 7)^2) - (0.00012828 \times \text{age})$

% Fat: Siri: 457/BD-414/2 (Ehrman, 2010; Siri, 1956)(Siri, 1956)

% Fat Brozek: 495/BD-450 (Brozek, J; Grande, F; Anderson, 1963; Ehrman, 2010)

Waist circumference, measured at the natural waist (around the smallest area of the abdomen), was measured using a tension-controlled tape. If measurements were within ¼" of each other, the average was reported. If the variance was greater than ¼" measurements were repeated until measurements were within ¼ inch of each other. This measure was used as a correlate for visceral adiposity (Ehrman, 2010). Hip circumference, measured around the largest area of the buttocks was used in conjunction with the waist circumference (WC) to calculate the WHR, by dividing waist circumference by hip circumference (Ehrman, 2010; Gregory Byron Dwyer; Shala E. Davis; American College of Sports Medicine (ACSM) Staff, 2008).Hip circumference

measurements were taken in duplicate and averaged. SAD was measured using a caliper to the nearest 0.1 cm at the umbilicus and between the 4th and 5th lumbar vertebrae on the exhale; two separate measurements were taken and then averaged(U. Risérus, De Faire, Berglund, & Hellénius, 2010a). SAD was measured in conjunction with the typical waist circumference and waist to hip ratio to ascertain a more comprehensive estimation of the subjects' visceral body fat distribution.

VO_2Max -Treadmill GXT -Condition A:

The subjects arrived rested, not having exercised within the past 24 hours prior to the visit. Weight was taken on SECA scale, Polar heart rate monitor was put on, and the subject rested for 15 minutes. During this time the researcher inquired about the subjects' physical activity including their typical mile pace to determine the speed at which to begin the graded exercise test (GXT).

The subject then had their blood drawn from an antecubital vein into EDTA tubes (lavender top); following a 15 minute rest period. Participants were strongly encouraged to drink water to help maintain plasma volume.

At least 5 min post blood draw, the exercise testing began. Condition A consisted of a graded maximal treadmill test, following Dr. Goldfarb's unpublished protocol in order to determine the subject's maximal oxygen consumption (VO_2max) running on a Quinton Q-Stress Treadmill; This protocol gave the subject a 3-5 minute warm-up based on the subject's comments and heart rate. The speed of the treadmill was gradually increased during this time to elicit a heart rate between 120-135beats per minute (bpm). At this time the subject was then asked to place the mouth piece and nose clips on and

oxygen uptake was analyzed using a one-way valve via PARVO Medics TrueOne 2400 Metabolic Measurement System calibrated to known gases prior to the test. The treadmill speed was increased each minute to bring the subjects' heart rate up to 135-145 bpm. The subject indicated ratings of perceived exertion (RPE) and heart rate (HR), ventilation (V_E) and oxygen consumption (VO_2) were continuously monitored throughout the protocol. Once they reached the proper heart rate the grade of the treadmill increased 2.5% each minute until the subject reached VO_2 max, or they indicated they wanted to stop. The researcher always asked if it was okay to increase the workload, which was increased every minute until the participant requested termination or the researcher saw a plateau in VO_2. The subject indicated their RPE on a chart by pointing to the number (6-20) in the last 15 seconds of each workload. The researcher also recorded respiratory exchange ratio (RER), VO2, carbon dioxide output (VCO_2), and V_E throughout the test.

The test was terminated if the subject requested to stop due to volitional fatigue or their VO_2 plateaued or there were any abnormal occurrences. To achieve a true VO_2max, three of the four criteria must have been met: 95% age-predicted maximum HR, RER >1.1, VO_2plateau, or RPE >19.The VO_2max test is considered vigorous intensity and thus, should have elicited an increase in blood glucose, causing a rise in insulin, which may have altered plasma apelin concentration. The rise in blood glucose was likely caused by a catecholamine response to high intensity exercise as well as glucagon increasing, allowing glucose to dump into the blood due to increased carbohydrate requirements, as carbohydrate provides more energy more rapidly at higher intensity of exercise. Water was provided prior, post, and with the one hour following the GXT to

ensure proper hydration. Blood was also taken immediately after the VO_2max test but was not utilized for this aspect of the study as this aspect focused on resting blood apelin.

As noted before, the subjects participated in three treatment conditions as part of a larger study, however only resting, pre-visit values will be reported. These three conditions were semi-randomized in that the subjects were randomly assigned to either Condition A (VO_2max) or Condition C (54 g glucose feeding) first. It was imperative that the VO_2max test occurred prior to the submaximal run, in order to provide a reference for the 70-75% submaximal treadmill run. Subjects either completed the study conditions as follows: Condition A, B, C; C, A, B; or A, C, B.

Blood Timing and Handling

Prior to intervention, following a≥15-minute rest period in the laboratory in order to obtain a baseline sample at rest in a fasted state at visits A, B and C. Blood samples were obtained from the antecubital vein using 7 mL EDTA tubes (lavender top) and immediately processed then centrifuged. EDTA, a substances used to prevent clotting via Calcium binding, was used because all previous studies have utilized EDTA tubes except 4 (2 did not specify, 2 used serum) as specified in the protocol for Phoenix Pharmaceuticals]. The remaining blood was placed in an ice bucket and immediately transported and centrifuged in a Beckman-Coulter Allegra swinging bucket centrifuge or a Beckman-Coulter Avanti J-E centrifuge for 10 minutes at 3,000 revolutions per minute (RPM) at 4° C to obtain plasma. The plasma samples were then aliquoted into plastic microtubes (to prevent freeze thawing of samples for different assays) and placed in an Ultima II freezer at -80°C for storage until analysis.

Blood Sampling & Assays:

Plasma apelin levels were measured using the ELISA immunoassay (EK-057-15) procedures described (Phoenix Pharmaceuticals) and measured at 450 nanometers (nm) using a BIO-TEK INSTRUMENTS PowerWave x340 KC Junior Microplate reader in duplicate. If the duplicates were not within 2 standard deviations from each other, the sample was measured again. The average of the two closest values was utilized to determine apelin concentration.

Statistical Analysis

Using the G*Power application, a sample size of n=11 was deemed appropriate for this study, yielding a power of ≥ 0.80. This power analysis was based on an effect size of r=0.7 and α=0.05 and a population mean and standard deviation of 0.438±0.191 ng/ml, determined from healthy controls in 7 preceding studies. In addition each subject's caloric balance was calculated using the Harris-Benedict equation to determine and compared to plasma apelin concentrations.

The data was analyzed using IBM SPSS using repeated-measures ANOVA (RMANOVA) within subject for each variable as well as nutrient data and plasma apelin concentration for each visit in order to compare across conditions to determine if there are variations for each subject. When no variations were found, the diets were collapsed across time points and reported as an average.

In addition, correlational analysis compared apelin to each of the outcome variables, using SPSS Pearson Correlational Analysis. If Mauchly's test for sphericity found that the data was not equally distributed, a Spearman's Correlational Analysis was

run. In addition, all measures were compared by gender with a simple t-test, to determine if there were gender differences. The significance level was set at 0.05 for all data analysis.

CHAPTER IV

RESULTS

The results of the current study are presented in this chapter. This chapter can be categorized into subject descriptive characteristics, body composition data, and nutritional data. The relationships of these factors with apelin will then be presented as well.

There were 15 subjects accepted for this study; each was required to report to the UNCG Exercise Physiology laboratory at a consistent time across conditions in the morning (between 6-9 am) in a resting, post-absorptive state. All subjects were apparently healthy and met the inclusion criteria. Three subjects were eliminated from inclusion as they demonstrated hypoglycemia at rest and/or a hypoglycemic response to the 54g oral glucose load. Therefore, the data in the results reflects the 12 subjects who met all of the criteria for inclusion, unless reported as (all subjects). Each subject successfully completed all treatment conditions, including achieving a true VO_2max (51.15 ±11.07 mL/kg/min) during a GXT, in accordance with established criteria (Midgley, McNaughton, Polman, &Marchang, 2007).

Subject Descriptive Characteristics

Table 14. Subject Descriptive Characteristics. Expressed in overall terms and by gender. Values shown are means ±standard deviation. *Significantly different between genders ($p<0.05$).

Subject Descriptive Characteristics			
Variable	***Overall***	***Males (n=7)***	***Females (n=5)***
Age (yrs.)	22.75 ± 2.96	22.43 ± 2.88	23.2 ± 3.35
Weight (kg)	68.56 ± 13.08	72.14 ± 15.11	63.54 ± 8.59
Height (cm)	171.04 ± 7.53	174.29 ± 5.82	166.5 ± 7.8
%BF	15.32±8.09	11.02±6.11	21.33±6.84
BMI	23.37±3.55	23.66±3.96	22.96±3.27
SAD (cm)	17.04±6.36	19.69±3.10	18.91±3.15
WHR	0.96±0.27	0.82±0.06	0.76±0.05
RHR (bpm)	65 ± 9.17	63.43 ± 9.07	67.2 ± 9.86
Resting SBP (mmHg)	119.67 ± 9.34	120.29 ± 9.83	118.8 ± 9.65
Resting DBP (mmHg)	77.83 ± 7.29	76.57 ± 8.04	79.6 ± 6.54
Resting MAP (mmHg)	91.78 ± 7.29	91.14 ± 8.51	92.67 ± 6.02
VO_{2max} (L/min)	3.49 ± 0.95	**4.02 ± 0.86***	**2.77 ± 0.47***
VO_{2max} (ml/kg/min)	51.14 ± 11.07	56.17 ± 10.32	44.09 ± 8.42
Apelin (ng/mL)	0.511 ± 0.333	0.464 ± 0.382	0.576 ± 0.239

Body Composition Factors

% BF was evaluated using both Siri &Brozek percent body fat calculations. In the graphs below, the red marker indicates a subject considered overweight but not obese in terms of % BF (Siri=23.36 %BF, Brozek=22.82 %BF), however, is considered overweight in terms of BMI (31.9 kg/m^2). The yellow marker indicates a subject that was considered obese using Siri % BF calculations (32.33 % BF), but overweight when using the Brozek equation (31.11 % BF); this subject however, was not considered obese in terms of BMI (27.8 kg/m^2).

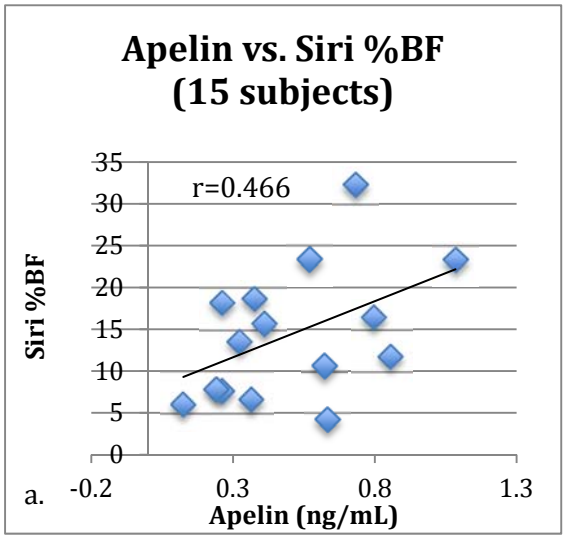

Figure 1a. Apelin vs. Siri %BF (15 subjects). The first figure (1a.)presents all 15 subjects' data (R^2=0.217, P=0.080), a non-significant relationship, however may be approaching significance.

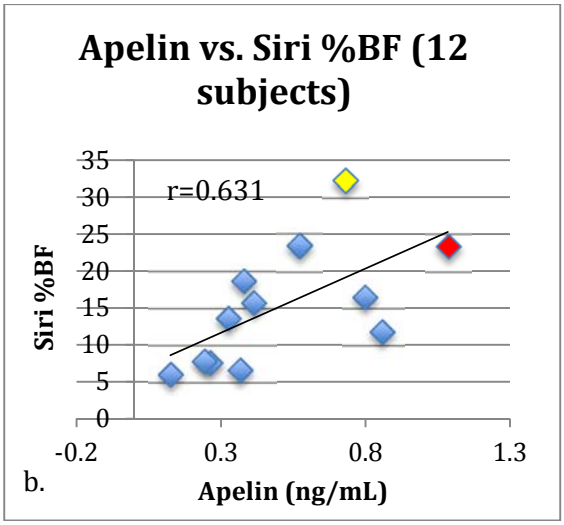

Figure 1b. Apelin vs. Siri %BF (12 subjects). The second figure (1b.) presents the 12 included subjects (R^2=0.399, P=0.028*), this relationship is significant at $p<0.05$ level.

The graphs above present the relationships between the subjects' plasma apelin concentrations at rest and their %BF calculated using the Siri equation. Figure 1a. shows the 12 included subjects and 1b. provides the reported data for all 15 subjects. Figure 1a indicates a significant correlation between apelin and Siri %BF (P=0.028), while the relationship is non-significant in figure 1b, however it does approach significance (P=0.080).

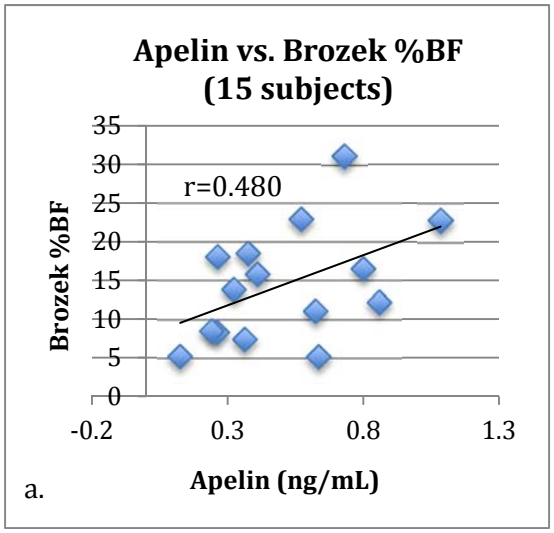

Figure 2a. Apelin vs. Brozek %BF (15 subjects). %BF was calculated using the Brozek equation for all 15 subjects (R^2=0.230, P=0.070), the relationship is non-significant, however does approach significance.

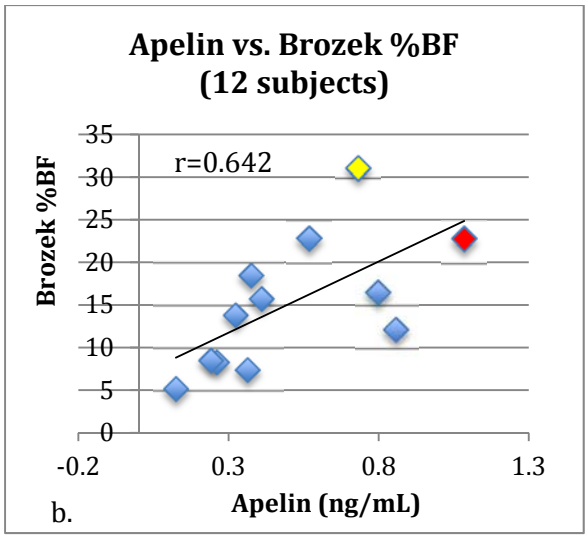

Figure 2b. Apelin vs. Brozek %BF (12 subjects). This figure presents the data for the 12 included subjects (R^2=0.412, P=0.024*), which is significant at the $p<0.05$ level.

The graphs above present the subjects' baseline plasma apelin concentration by their %BF Brozek formula. Figure 2a. presents the 12 included subjects and indicates a significant positive relationship between plasma apelin concentration and Brozek %BF (P=0.024), whereas figure 2b. presents all 15 subjects' data and was non-significant (P=0.070), however did approach significance ($p<0.05$).

The calculated values between Siri and Brozek %BF were similar, thus producing similar R^2 values and graphical representations. In the graph below, Siri and Brozek calculations are both presented on the same graph to display the similarities between the calculations.

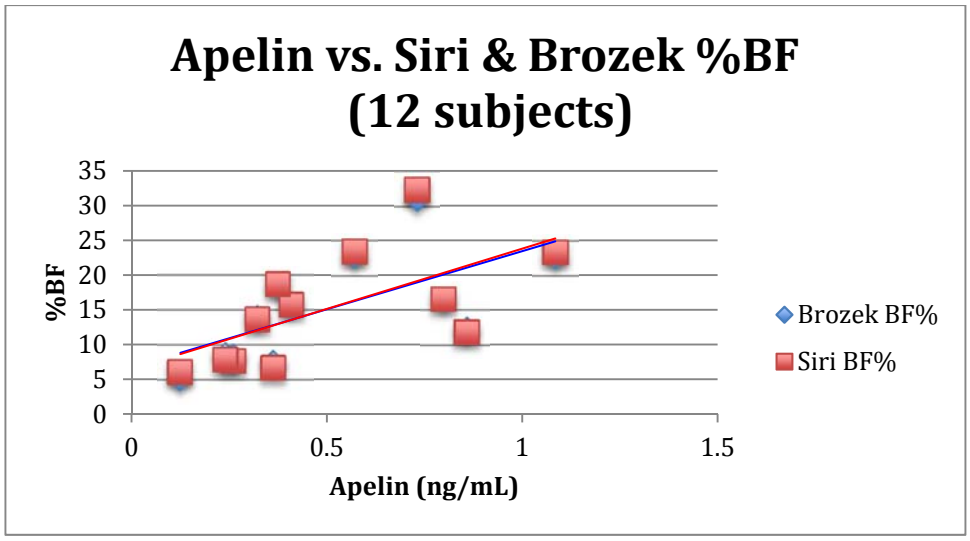

Figure 3. Apelin vs. Siri & Brozek %BF (12 subjects). This figure presents %BF calculated using both equations for the 12 included subjects.

This chart presents baseline plasma apelin concentrations versus both Siri and Brozek calculated % body fat for all 12 subjects. It is clear that the values do not deviate significantly from one another. The R^2-value for Siri % body fat is 0.399 ($p=0.028$) and the R^2-value for Brozek % body fat is 0.412 ($p=0.024$). Both of these indicate significant positive correlations between baseline plasma apelin concentrations and % BF of the subjects at the $p<0.5$ significant level.

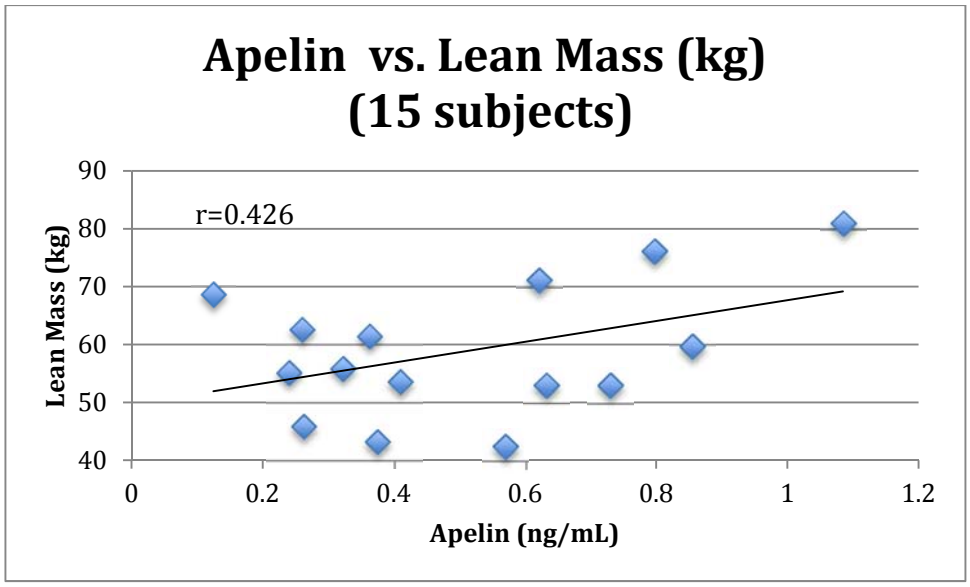

Figure 4. Apelin vs. Lean Mass (kg) (15 subjects). The relationship was non-significant (R^2=0.181, P=0.114).

This figure presents the subjects calculated lean body mass (kg) versus their resting plasma apelin concentration (ng/mL). It appeared that body composition (BMI/% BF) had stronger correlations with apelin than body fat distribution. In order to look at fat mass versus lean mass, the subjects' lean body mass was calculated using their average % BF calculation. No significant relationship was found (R^2=0.181, P=0.114).

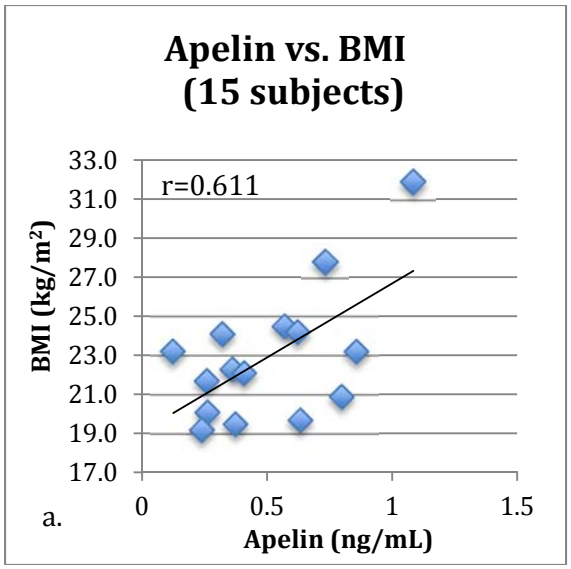

Figure 5a. Apelin vs. BMI (15 subjects). Figure 5a presents apelin versus their BMI (R^2=0.373, P=0.016*) indicating a significant positive correlation at the p<0.05 significance level.

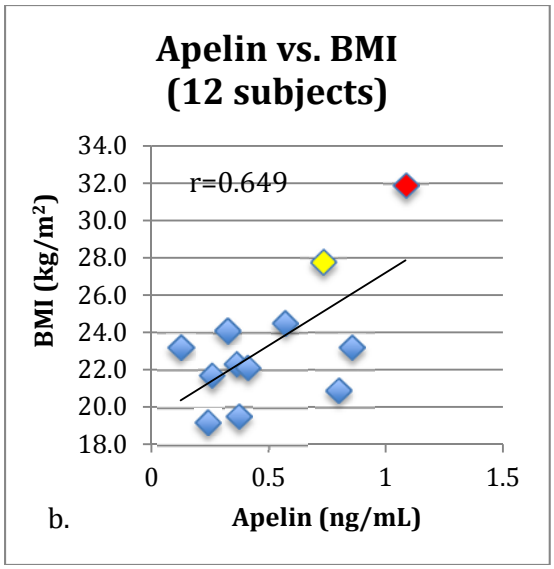

Figure 5b. Apelin vs. BMI (12 subjects). This figure presents the 12 reported subjects apelin vs. BMI relationship, which is also significant at p<0.05 level (R^2=0.412, P=0.022*).

In addition to %BF, BMI was calculated. Figure 4a presents the 12 included subjects and indicates a significant positive relationship between plasma apelin concentration and BMI (kg/m^2) (P=0.022). In addition, figure 2b presents a similar correlation, in which all 15 subjects data was included in analysis, also indicating a significant positive linear correlation (P=0.016).

Together these graphs and tables indicate that there is a positive linear relationship between plasma apelin concentration and % BF. Using BMI and % BF as indices, both showed significant positive linear relationships at the p<0.05 significance level. Using this data, the hypothesis stating that a positive linear relationship will occur between plasma apelin concentration and % BF can be accepted.

Table 15. Pearson Correlations of Anthropometric Measures. This table displays the correlations between anthropometric measurements and baseline plasma apelin concentrations. Siri %BF (0.028) and Brozek %BF (0.024), BMI (0.022) and SAD (0.029) were all found to be significant via Pearson's 2-tailed analysis ($p<0.05$). *Correlation is significant at the 0.05 level (2-tailed)

Pearson Correlations of Anthropometric Measures		
	Pearson Correlation	Significance
Siri %BF	**0.631***	**0.028**
Brozek %BF	**0.642***	**0.024**
BMI	**0.649***	**0.022**
Sagittal (cm)	**0.628***	**0.029**
WHR	0.178	0.579
WC (cm)	0.555	0.061

Body Fat Distribution Factors

In the graphs below, the red marker indicates a subject considered overweight but not obese in terms of %bf (Siri=23.36 %BF, Brozek=22.82 %BF), however, is considered overweight in terms of BMI (31.9 kg/m^2). The yellow marker indicates a subject that was considered obese using Siri %BF calculations (32.33 %BF), but overweight when using the Brozek equation (31.11 %BF); this subject however, was no considered obese in terms of BMI (27.8 kg/m^2).

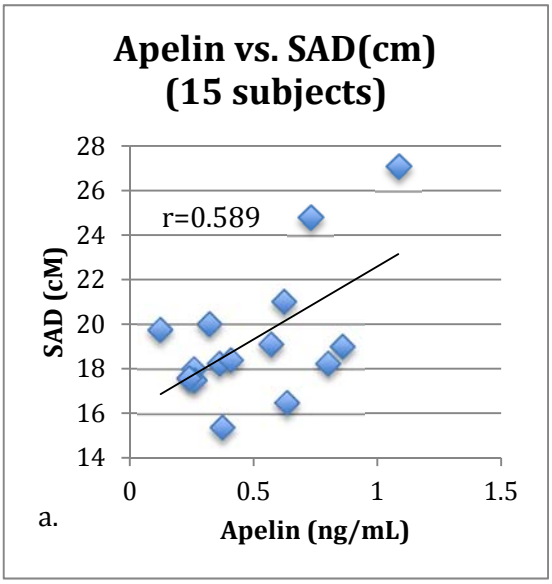

Figure 6a. Apelin vs. SAD (cm) (15 subjects).Figure 6a.presents SAD vs. apelin for all 15 subjects (R^2=0.347, P=0.021*) indicating a significant positive relationship at the p<0.05 significance level.

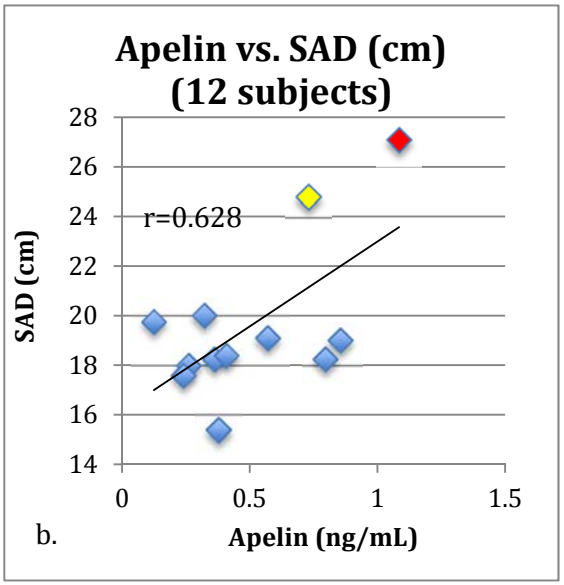

Figure 6b. Apelin vs. SAD (cm) (12 subjects). This shows the 12 included subjects' baseline plasma apelin concentration vs. SAD (R^2=0.394, P=0.029*) indicating a significant positive correlation at p<0.05 level.

The figures above present SAD versus baseline plasma apelin concentration. Figure 5a presents the data for the included 12 subjects and indicates a significant positive linear relationship between SAD and plasma apelin (P=0.029). Similarly, figure 5b. presents SAD versus plasma apelin for all 15 subjects and indicates a significant positive linear correlation (P=0.021).

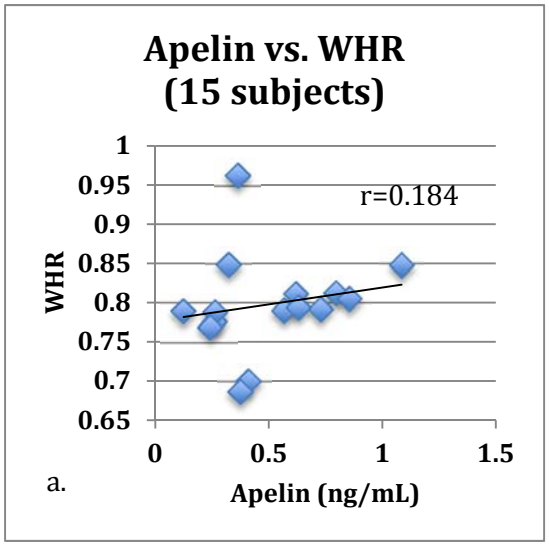

Figure 7a. Apelin vs. WHR (15 subjects). Figure 7a presents WHR versus apelin for all 15 subjects (R^2=0.034, P=0.511) this relationship is non-significant at p<0.05 level.

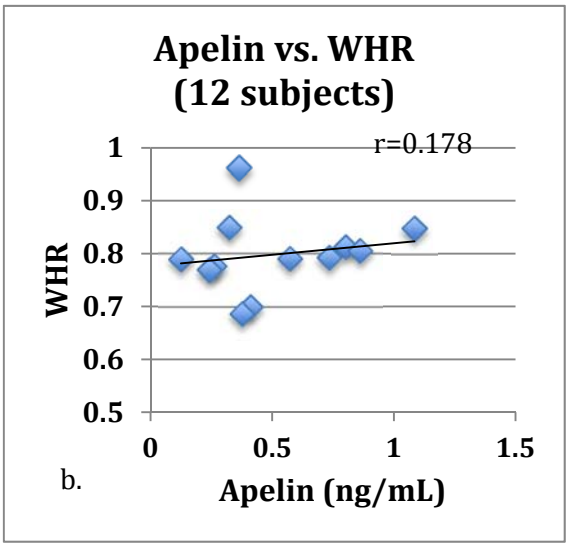

Figure 7b. Apelin vs. WHR (12 subjects). Figure 7b. presents the 12 included subjects' apelin concentration vs. WHR; the relationship is non-significant at the p<0.05 significance level (R^2=0.032, p=0.579).

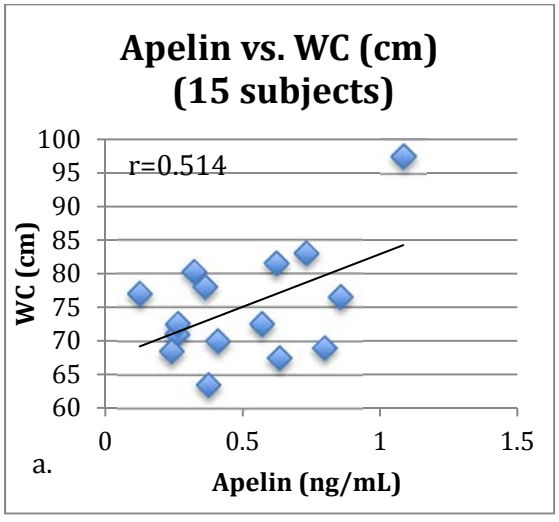

Figure 8a. Apelin vs. WC (cm) (15 subjects). It is non-significant (R^2=0.264, P=0.050), however does approach significance.

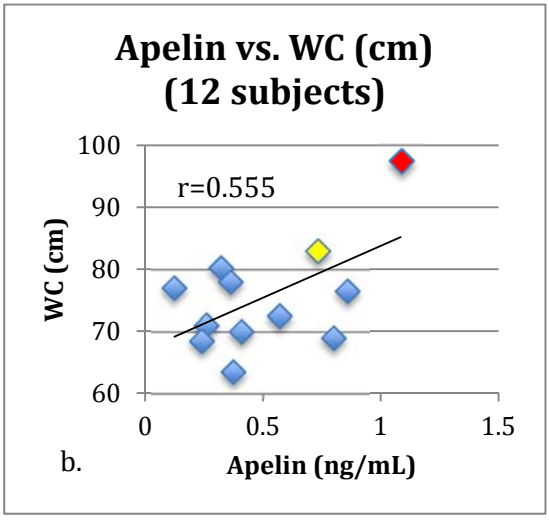

Figure 8b. Apelin vs. WC (cm) (12 subjects). Presents the 12 included subjects, the relationship was non-significant (R^2=0.308, p=0.061), approaches significance at p<0.05 level

No significant relationship was found between WHR and apelin in either group. The WC measurements of the 12 subjects studied has a small range and indicated a positive linear relationship approaching significance (R=0.555, p=0.061) at the $p<0.05$ significance level. However, when all 15 subjects were included, the relationship was significant (P=0.050).

Participant Food Consumption

Table 16. Mean Values for Nutritional Data. This table presents the average daily nutritional data for the subjects collapsed across all 3 time points, providing the mean and standard deviation. The data is also divided into male and female subjects.

Mean Values for Nutritional Data			
	Group Mean ± SD	***Males (n=7)***	***Females (n=5)***
Total kCal	2161.54±544	2446.18±989.10	1763.06±518.92
CHO (g)	262.18±111.61	302.51±126.18	205.72±59.54
CHO (kCal)	1048.72±446.46	1210.04±504.72	822.87±238.15
Protein (g)	99.25±40.42	111.97±46.37	81.43±24.21
Protein (kCal)	396.99±161.66	447.89±185.48	325.72±96.84
Total Fat (g)	81.62±40.14	89.28±46.34	70.88±31.04
Total Fat (kCal)	734.59±361.26	803.56±417.02	637.96±279.34
Vitamin A (mcg)	473.86±264.15	548.86±316.42	368.85±134.45
Vitamin C (mg)	98.41±101.03	122.65±130.59	64.48±4.17
Vitamin E (mg)	13.25±3.49	13.32±2.90	13.16±0.85
Zinc (mg)	9.40±0.68	9.57±0.53	9.17±0.85

Macronutrients

Table 17. Average Daily Macronutrient Intake vs. Recommended. This table presents the subjects' actual average macronutrient intake in terms of % of total kCal intake and also presents the recommended average macronutrient intake as a percentage of total kCal consumption.

Average Daily Macronutrient Intake vs. Recommended		
Macronutrient	**% Range (Actual)**	**% Range (Recommended)**
Fat	26-44%	20-35%
Protein	11-28%	10-35%
Carbohydrates	42-57%	45-65%

Table 18. Percent Macronutrient Intakes of Total kCal. The reported percentage of total calories consumed for each macronutrient pooled across visits.

Percent Macronutrient Intakes of Total kCal				
Subject	**Gender**	**%CHO**	**%Protein**	**%Fat**
1	Male	57.65%	21.26%	26.06%
2	Female	49.25%	17.22%	35.42%
3	Female	47.27%	16.67%	37.85%
4	Female	41.89%	20.99%	44.63%
6	Male	46.11%	26.62%	27.67%
7	Male	50.48%	11.22%	37.77%
8	Male	52.13%	18.53%	28.92%
11	Male	35.95%	27.88%	35.50%
12	Female	52.67%	16.06%	29.83%
13	Male	53.09%	14.19%	27.94%
14	Female	43.24%	22.09%	28.02%
15	Male	47.31%	16.62%	40.86%

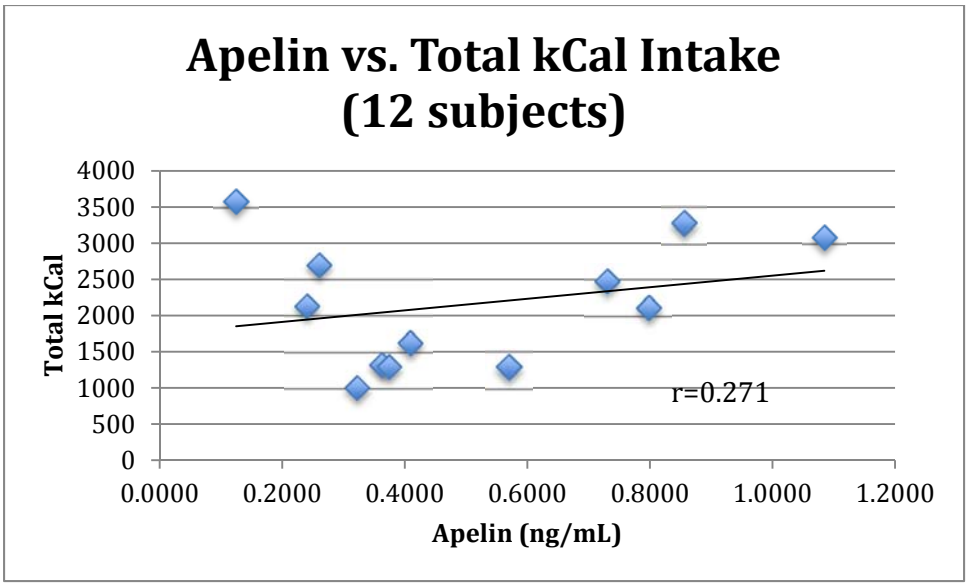

Figure 9. Apelin vs. Total kCal Intake. This figure presents the 12 reported subjects total reported kCal intake versus plasma apelin concentration (R^2=0.073, P=0.395) indicating no significant relationship.

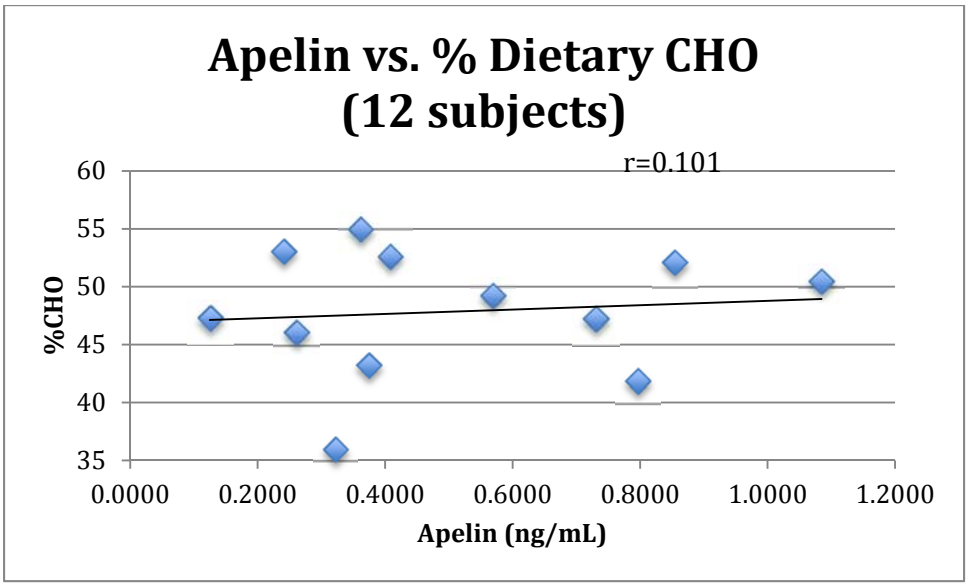

Figure 10. Apelin vs. % Dietary CHO. This figure presents the 12 reported subjects' reported %CHO intake by plasma apelin concentration (R^2=0.010, P=0.755), no significant relationship was found.

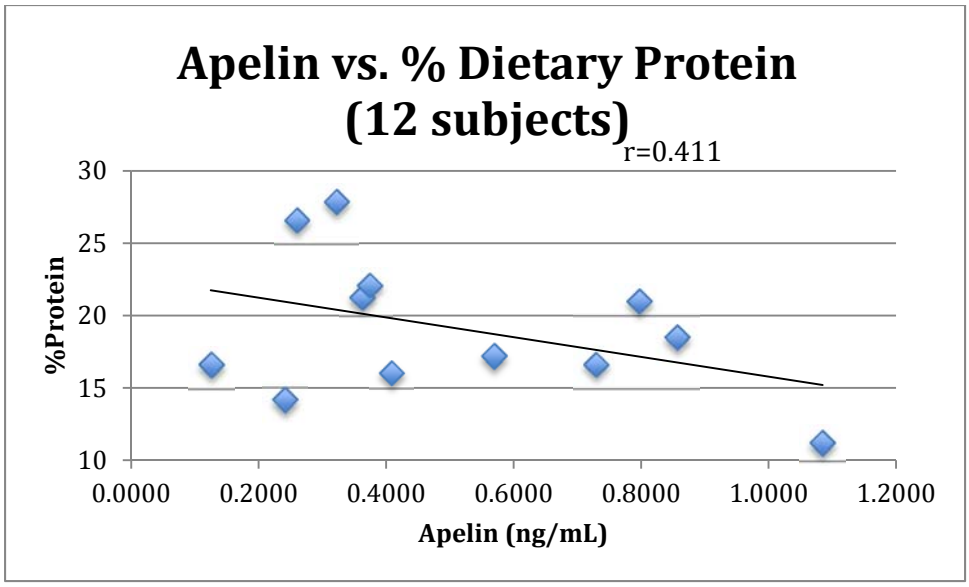

Figure 11. Apelin vs. % Dietary Protein. This figure presents the subjects reported protein intake as a percentage of their total kcal intake versus plasma apelin (R^2=0.169, P=0.185). A slightly negative linear relationship is seen, however not significant.

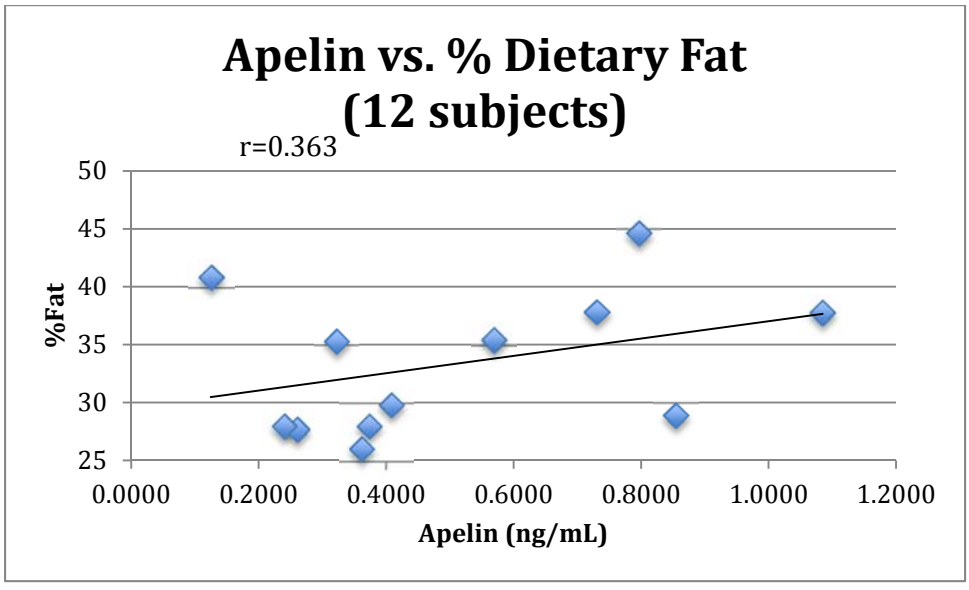

Figure 12. Apelin vs. % Dietary Fat. The figure above presents the subjects' reported fat intake as a percentage of their total kcal intake versus plasma apelin concentration (R^2=0.132, P=0.247). A slightly positive linear relationship can be seen, however is not significant.

Table 19. Mauchly's Test of Sphericity for Dietary Factors. The data indicates that sphericity was found for all nutritional variables analyzed.

Mauchly's Test of Sphericity for Dietary Factors					
Source	***Mauchly's W***	***Approx. Chi-Square***	***df***	***Sig.***	***Greenhouse-Geisser***
Total kCal	0.926	0.767	2	0.681	0.931
CHO	0.926	0.770	2	0.680	0.931
Protein	0.937	0.649	2	0.723	0.941
Total Fat	0.952	0.496	2	0.780	0.954
Vitamin A	**0.495**	**7.037**	**2**	**0.030***	**0.664**
Vitamin C	0.627	4.669	2	0.097	0.728
Vitamin E	0.601	5.100	2	0.078	0.715
Zinc	0.697	3.607	2	0.165	0.768

The data provided in the table presented above suggests that there is sphericity found with each variable except for Vitamin A, indicating a normal distribution. Because sphericity was found with each variable, a test of within-subjects effects was performed to determine if there were any significant relationships, and none were found. Mauchly's test of sphericity did not show normal distribution for Vitamin A, therefore a Spearman's correlational analysis was done. No significance was found with Spearman's correlational analysis.

Table 20. Tests of Within-Subjects Effects of Dietary Factors**.** Since sphericity was assumed for all variables using Mauchly's Test of Sphericity, a test of within-subjects effects was done for each variable. No significance was found.

Tests of Within-Subjects Effects of Dietary Factors					
Source	***Type III Sum of Squares***	***df***	***Mean Squares***	***F***	***Sig.***
Total kCal	100705.92	2	50352.9	.402	0.674
Error (kCal)	2754193.15	22	125190.6		
CHO	7825.33	2	3912.66	1.221	0.314
Error (CHO)	70505.30	22	3204.8		
Protein	933.64	2	466.8	1.601	0.224
Error (Protein)	6413.96	22	291.5		
Fat	159.95	2	80	0.351	0.708
Error (Fat)	5005.8	22	227.5		
Vitamin A	571483693392	2	285741846696	0.601	0.557
Error (Vit A)	10458702789500	22	475441035886		
Vitamin C	2562.53	2	1281.26	0.217	0.807
Error (Vit C)	129874.6	22	5903.39		
Vitamin E	387.95	2	193.98	1.291	0.295
Error (Vit E)	3306.26	22	150.29		
Zinc	9.28	2	4.64	0.297	0.746
Error (Zinc)	343.12	22	15.6		

The data above suggest that assuming normal distribution for each factor besides vitamin A, there were no significant differences between the average daily nutritional intakes of each subject between the three time points. Because the subjects did not have significantly different dietary intakes at each visit, the dietary data was collapsed across time points, providing an average intake for each variable across all time points.

Antioxidants

This section presents the subjects' antioxidant and micronutrient intake in relation to baseline plasma apelin levels. Each micronutrient (Vitamin A, C, and E) were analyzed individually then pooled to create an average daily total micronutrient antioxidant intake and their average % of RDA consumed daily. The outliers are highlighted in red.

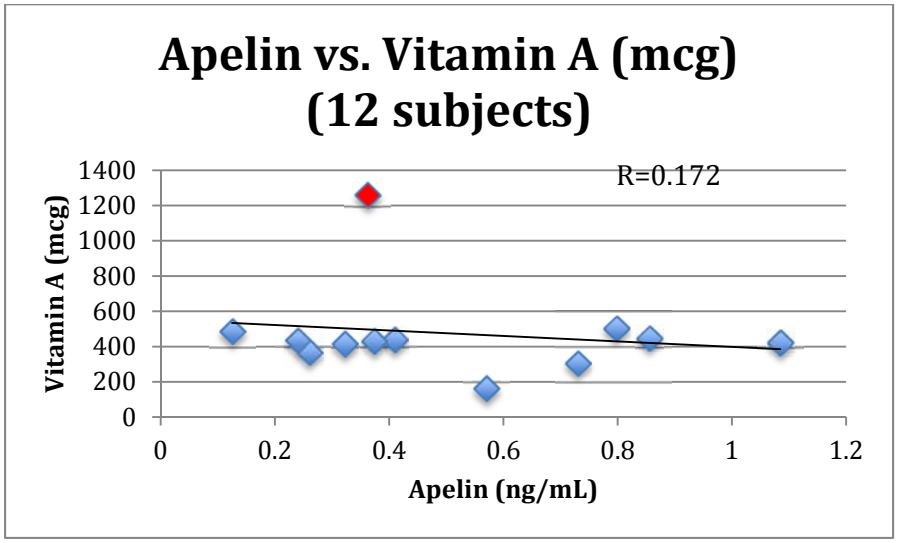

Figure 13. Apelin vs. Vitamin A (mcg). This graph presents the subjects' baseline plasma apelin concentration in relation to their average daily Vitamin A intake (mg). The data indicates that the relationship is not significant (R^2=0.030, =0.592). The red marker is an outlier for normal vitamin A intake.

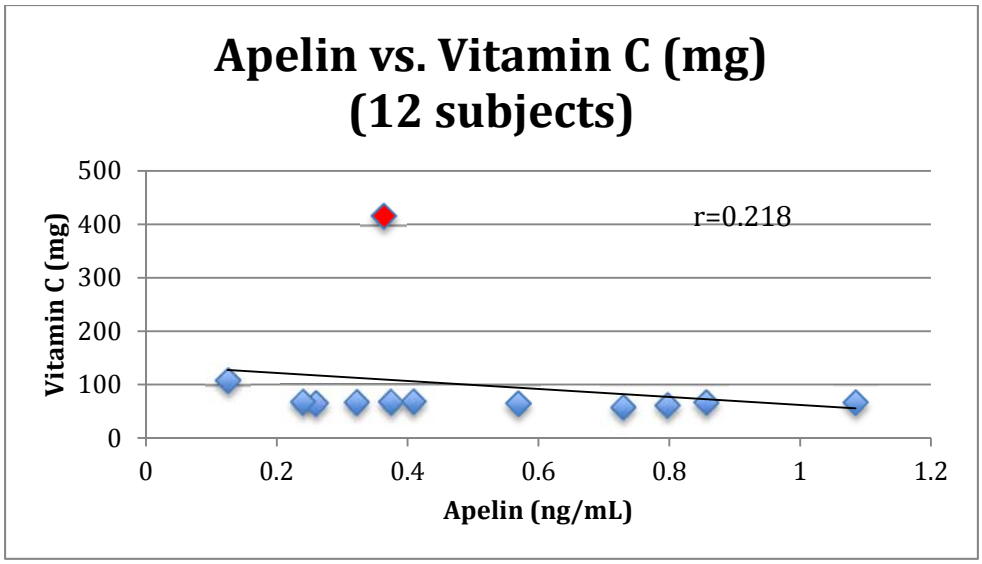

Figure 14. Apelin vs. Vitamin C (mg). This graph presents the subjects' baseline plasma apelin concentration in relation of average daily Vitamin C intake (mg). This relationship is weak and not significant (R^2=0.048, P=0.496). The red marker is an outlier for normal vitamin C intake.

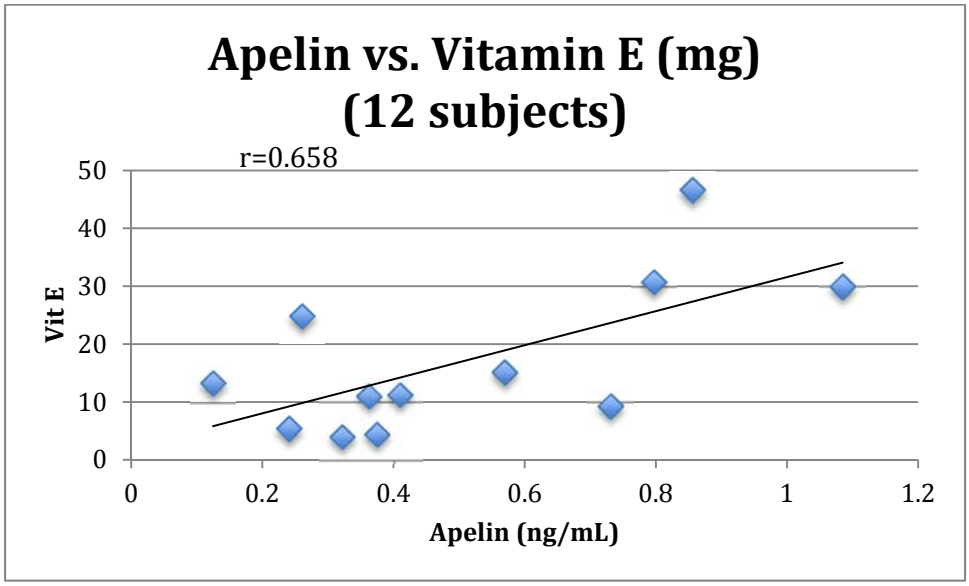

Figure 15. Apelin vs. Vitamin E (mg)**.** This graph shows the baseline plasma apelin concentration versus the subjects' average daily Vitamin E intake. The data indicates a significant positive linear realtionship between vitamin E intake and plasma apelin (R^2=0.433, P=0.020*)

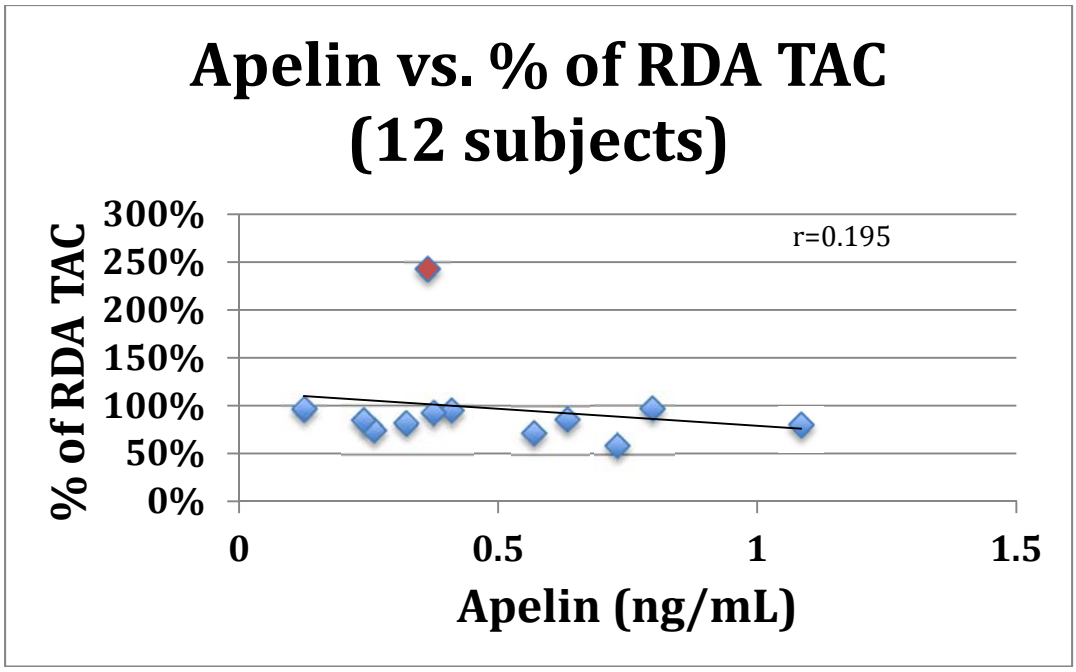

Figure 16. Apelin vs. % of RDA TAC.

This figure presents the subjects' baseline plasma apelin level by % of total antioxidant consumption (TAC). The % RDA consumed was calculated by summing the vitamin percentage of A, C, and E consumed compared to the RDA. The data indicates a weak negative linear correlation that is not significant (R^2=0.038, P=0.544). The red marker is an outlier for normal vitamin total intake.

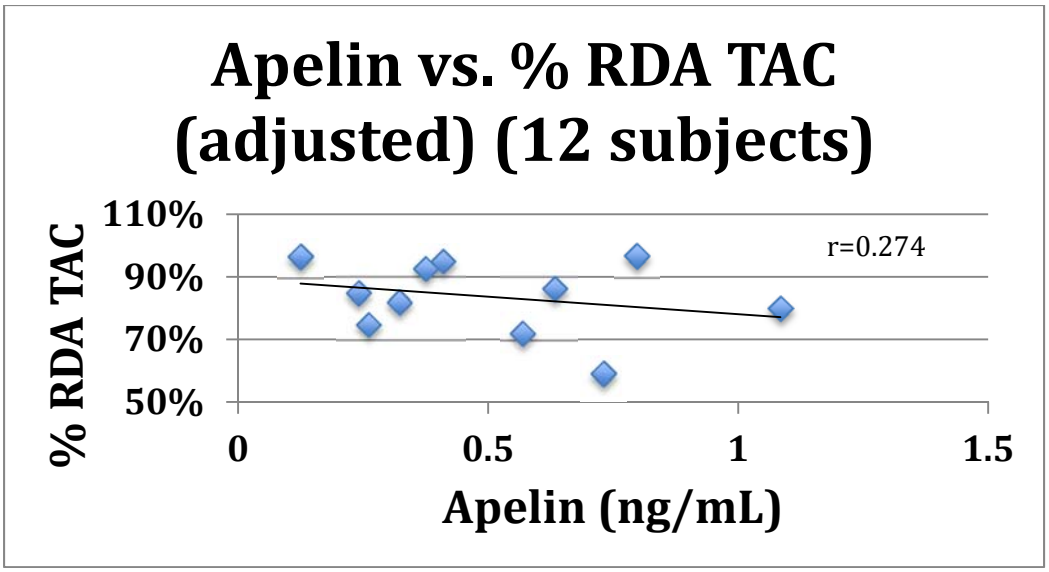

Figure 17. Apelin vs. % RDA TAC (adjusted). This graph presents the same data as above without the outlier. There was one participant who reported consuming >200% of the RDA for total antioxidant content. When removed the range of data is significantly smaller, however there was still no significant effect (R^2=0.075, P=0.415).

Table 21. Pearson Correlations of Micronutrient Factors.This table presents the pearson correlations of each micronutrient variable in terms of apelin.

Pearson Correlations of Micronutrient Factors			
	R-value	**R^2-value**	**Significance**
Vitamin A (mcg)	0.172	0.030	0.592
Vitamin C (mg)	0.218	0.048	0.496
Vitamin E (mg)	0.658	0.433	0.020*
Zinc (mg)	0.024	0.001	0.940
% RDA	0.195	0.038	0.544
% RDA (adjusted)	0.274	0.075	0.415

Zinc

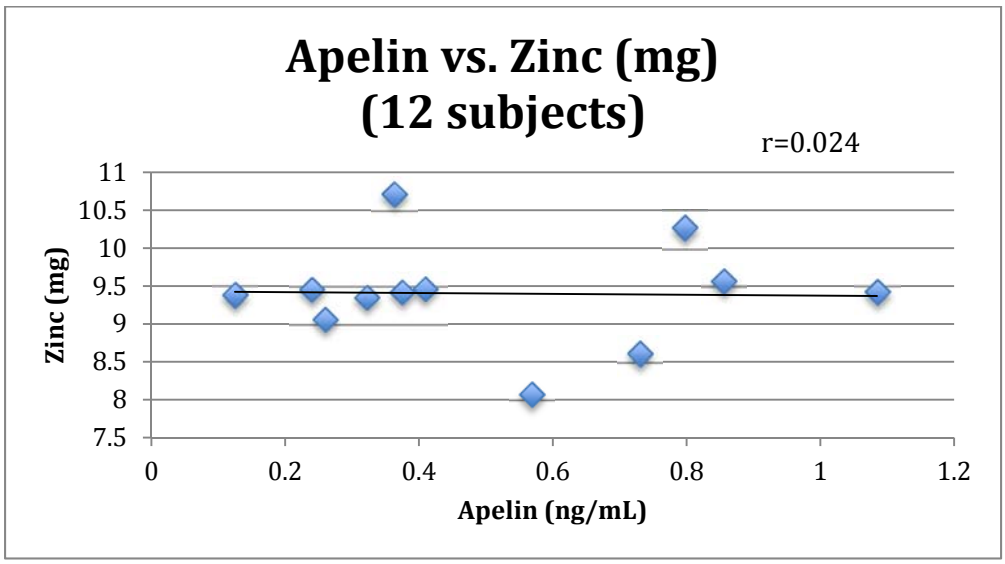

Figure 18. Apelin vs. Zinc (mg)**.** This graph presents the baseline plasma apelin concentration (ng/mL) versus average daily Zinc intake (mg). No significance was found (R^2=0.001, P=0.940).

It was hypothesized that higher zinc intake would illicit lower plasma apelin levels at baseline, however the data shows that there was no significant relationship between zinc intake and baseline plasma apelin concentrations in the studied cohort. Therefore, we cannot accept or reject the hypothesis stating that increased zinc intake would decrease plasma apelin concentrations in healthy, young adult subjects. Further research should be done using larger cohort and possibly using diet intervention.

Apelin

Table 22. Resting Plasma Apelin Concentrations. This table presents the resting, fasted plasma apelin concentrations of each subject at each visit.

Resting Plasma Apelin Concentrations			
Subject	**Visit 1**	**Visit 2**	**Visit 3**
1	0.393	0.270	0.424
2	0.627	0.578	0.503
3	0.784	0.575	0.832
4	0.564	1.186	0.641
5	0.268	0.2235	0.290
6	0.649	1.228	1.377
7	0.907	0.714	0.945
8	0.505	0.2435	0.2180
9	0.701	0.332	0.193
10	0.112	0.364	0.246
11	0.582	0.304	0.237
12	0.112	0.109	0.1535

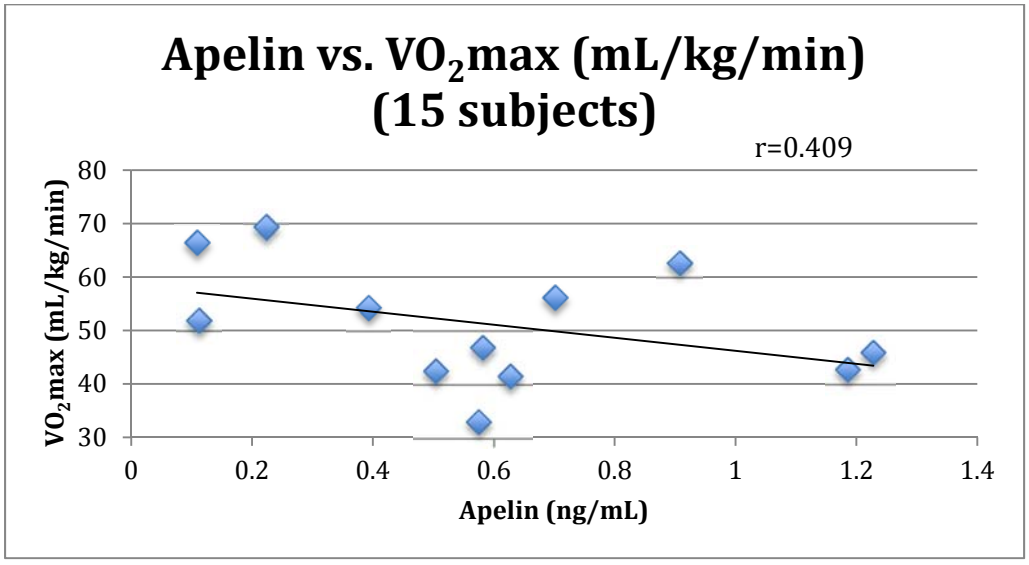

Figure 19. Apelin vs. VO_2max (mL/kg/min). This figure presents the 15 subjects' baseline plasma apelin by maximal oxygen consumption. Is it fitness or fatness?

This figure presents the subjects' VO_2max versus baseline plasma apelin concentration (R^2=0.186, P=0.186) indicating a weak negative relationship.

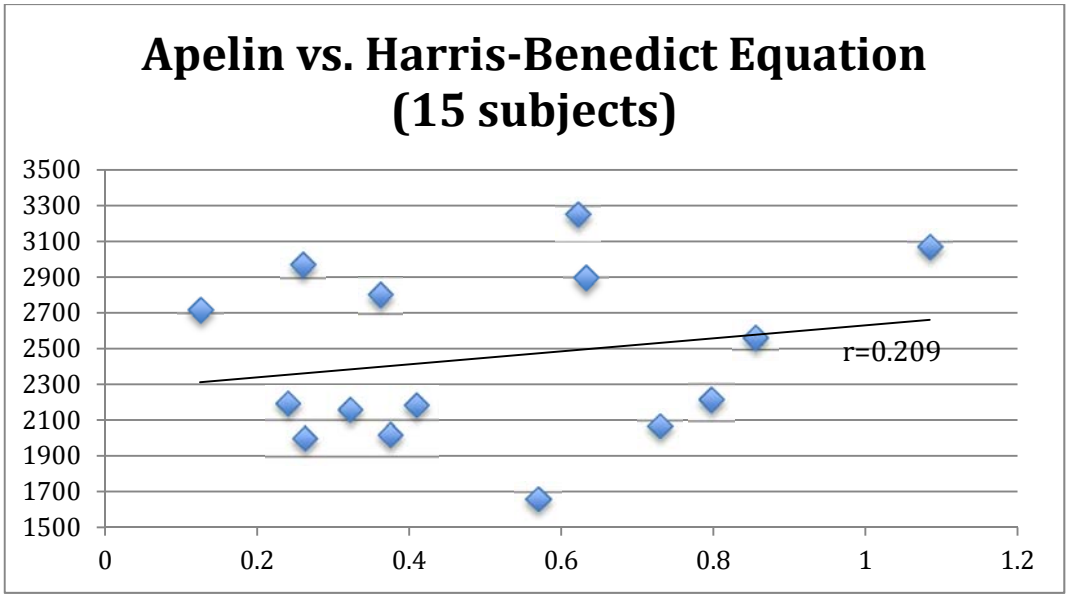

Figure 20. Apelin vs. Harris-Benedict Equation. Non-significant (R^2=0.044, P=0.454).

In order to determine the caloric balance of the subjects studied, the subjects' basal metabolic rate was calculated and adjusted for physical activity, then analyzed in relation to their plasma apelin concentration fasting and at rest.

CHAPTER V

DISCUSSION

This chapter provides insight into the results of the presented study as well as compares the results to the literature, as well as suggests future directions. The primary purposes of this study were to evaluate whether diet and/or body composition influence resting plasma apelin concentration. An additional aim was to establish a baseline resting plasma apelin concentration for healthy young adults, as much of the research on apelin has been focused on clinical populations. It is important to establish a proper baseline plasma apelin concentration for healthy populations, as it is difficult to analyze outcomes of exercise and interventional studies without an appropriate baseline against which to compare apelin concentration. Subjects were recruited on a volunteer basis, the majority being undergraduate students in the Kinesiology department at UNC-Greensboro. This may explain the skewed VO_2max scores in which all subjects were above average, with most subjects in the excellent to superior range for their age and gender. This indicates our subject group was more fit than the average person in their age group and gender. In order to analyze this we looked at the subjects VO_2max scores versus their plasma apelin concentration and no significant relationship was found.

The subjects had normal weight and height for this age group (22.75±2.96 years). The average %BF (15.32±8.09%; M=11.02±6.11, F=21.33±6.84%) and BMI (23.37±3.55 kg/m^2) of the subjects was normal respective to their gender (based on CDC

norms). One female subject was considered overweight via BMI and obese in terms of %BF. In addition, one male subject was considered obese I via BMI, however was considered only overweight in terms of %BF.

The subjects had a normal resting heart rate (65±9.17 bpm) and normal systolic and diastolic blood pressures (119.67±9.34 mmHg SBP; 77.83±7.29 mmHg DBP). The maximal oxygen consumption (51.14±11.07 ml/kg/min) is considered superior for their age group; each subject was in the excellent to superior range based on ACSM values (Ehrman, 2010).

Body Composition

The first proposed hypothesis predicted that a positive relationship would exist between percent body fat and plasma apelin concentration at rest. To evaluate the subjects' % BF, 7-site skinfold analysis was performed upon the first visit. Both Siri and Brozek %BF calculations were used and not found to be significantly different from one another. Both Siri %BF ($P=0.028$) and Brozek ($P=0.024$) calculations showed a significant positive linear correlation with baseline plasma apelin concentration at $p<0.05$ significance level. While DEXA is the gold standard and should be looked at in the future, we were limited to the skinfold analysis method to establish % BF in individuals. BMI, which is a preferable index for populations, was also examined.

BMI is a widely used and accepted form of classifying subjects as overweight and obese, however it is generally use as a population index. BMI is used more clinically to categorize populations. Similar to Siri and Brozek %BF, the data on BMI compared to resting apelin also revealed a significant positive linear relationship ($r=0.649$, $P=0.022$) at

$p<0.05$ significance level. Surprisingly, this relationship is slightly stronger than both Siri and Brozek %BF, indicating that all three measurements may be used as a predictor of resting plasma apelin concentrations. This data is supported by previous literature in which, other studies found that as %BF and BMI increased, so did apelin, indicating there is a positive relationship between these factors and resting apelin concentrations. (H. J. Ba et al., 2014; Daviaud et al., 2006; Heinonen et al., 2009; Sheibani et al., 2012). .

While BMI is typically used as an index for population based studies, and % BF is typically considered more accurate for individual. The data in this study indicated that BMI and %BF are both good indicators for predicting resting plasma apelin levels, with BMI having a slightly stronger relationship with apelin than %BF.

Body Fat Distribution

In addition to 7-site skinfold measurements and BMI several other measurements were taken prior to the first treatment condition on their first visit to the lab. SAD and WHR were measured to establish the subjects' body fat distribution.

The data collected shows a significant positive linear correlation between plasma apelin concentration at rest and SAD (cm) ($r=0.628$, $P=0.029$) at the $p<0.05$ significance level. To further support this finding, the strength of the correlation increases when SAD is analyzed for all 15 subjects ($r=0.589$, $P=0.021$). To our knowledge this is the first study which reports a significant relationship between apelin and visceral obesity (SAD and WC). This is important because while several studies have reported significant relationships between BMI and %BF and apelin, this study indicates that the location of body fat in addition to proportion of body fat may influence apelin concentration.

SAD is a direct measurement of visceral adiposity. A second measure of visceral adiposity, WC, was analyzed. In addition, WHR was established for each participant, which is an indicator of increased cardiometabolic risk in relation to visceral obesity. SAD has been suggested to be a stronger indicator of body fat distribution for visceral obesity than WHR (Ohrvall, Berglund, & Vessby, 2000; U. Risérus et al., 2010a; Ulf Risérus et al., 2004)which is controversial. It has been suggested that gender and amount of obesity may be a factor towards increased cardiometabolic risk due to visceral obesity(U. Risérus et al., 2010a).

Several studies have examined SAD in terms of assessing metabolic risk. SAD has been proved an accurate indicator of visceral fat area on the L4-L5 level when compared to multiple scans compared to MRI (Kvist, 1988; U. Risérus et al., 2010a). Several studies have shown SAD to be a better indicator of CVD risk and strongly associated with glucose intolerance, mortality, cholesterol, HTN, and general risk for metabolic disorders independent of BMI (Kvist, 1988; Ohrvall et al., 2000; U. Risérus et al., 2010a; U. Risérus, De Faire, Berglund, & Hellénius, 2010b; van der Kooy, Leenen, Seidell, Deurenberg, & Visser, 1993). However, contradictory evidence has been published indicating that SAD is only comparable to WC and WHR as an indicator of visceral fat in obese men, whereas WC and WHR are reported to be superior to SAD when assessing visceral fat in women (Kvist, 1988; van der Kooy et al., 1993)

While not an ideal measure, WHR was also analyzed; the reported results indicate that the relationship is not significant ($r=0.184$, $P=0.579$) at the $p<0.05$ significance level. A better measure of visceral adiposity, WC is a commonly used indicator of body fat

distribution, and in conjunction with BMI, WC may predict increased risk for metabolic dysfunction in normal and overweight populations (National Heart Lung and Blood Institute & National Institutes of Health (NIH) National Heart, Lung, and Blood Institute, 1998). The data collected shows a positive linear correlation between WC, however not significant ($p<0.05$), it is approaching significance ($r=0.555$, $P=0.061$). These results suggest that WC may be a good correlate to support the hypotheses regarding body fat distribution. Interestingly, when all 15 subjects are included, the relationship between plasma apelin concentration and WC becomes significant ($r=0.514$, $P=0.050$).

The data collected regarding body fat distribution suggests that as visceral adiposity increases, so does plasma apelin concentration at rest. While, only SAD had a significant positive linear relationship with plasma apelin concentrations at rest ($r=0.628$, $P=0.029$*), the relationship between WC and apelin concentrations at rest approached significance ($r=0.555$, $P=0.061$) at the $p<0.05$ significance level. Interestingly, when all 15 subjects were included in analysis, the relationship between WC and plasma apelin becomes significant ($r=0.514$, $P=0.050$) and SAD had an even stronger relationship than the 12-subject correlation ($r=0.589$, $P=0.021$). In contrast, the relationship between WHR and plasma apelin concentrations at rest was non-significant ($r=0.178$, $P=0.579$), and was still non-significant when all 15 subjects were included.

According to the data collected, the hypothesis stating that subjects exhibiting increased visceral obesity would have increased baseline plasma apelin concentrations can be accepted. While the strength of the relationships varied dependent on the measure analyzed, all three indicated a positive linear correlation between visceral obesity and

plasma apelin concentrations at rest. Several studies have proven associations between plasma apelin concentrations and BMI, however few of these studies reported WC, WHR, or SAD to predict visceral adiposity. Unfortunately these studies only reported WC (cm) in the descriptive characteristics tables and did not report relationships between WC and plasma apelin concentrations (Heinonen et al., 2009; Soriguer et al., 2009).

Macronutrient Consumption

In terms of macronutrients, our subjects reported diets similar to those reported by the USDA NHANES study from 2011-2012 in "What We Eat in America." On average, the subjects reported consuming about 2,049 total kCal per day, which is in line with the reported 2191 kCals per day consumed by the average American as reported by NHANES, 2011 as well as the 2,000-3,000 kCal diet recommended by the USDA/USHHS. In general, the participants were active and healthy, eating well-rounded diets with an in-depth education about nutrition, according to their PAR-Q and pre-participation questionnaires.

The third hypothesis stated that individuals consuming a high fat diet would have increased plasma apelin concentration at rest. Only 3 subjects had diets with considered high in fat (>35% of total kCal) and had varied confounding factors that may have influenced the diet relationship with plasma apelin. Two of the subjects were considered highly active, where as the one sedentary subjects had higher plasma apelin levels when compared to the two active subjects. The relationship of high fat diets and apelin could not be analyzed with only three subjects as there are not enough subjects to power the analysis. It was important to look at macronutrient intake because animal studies have

shown that a HFD will induce maximal insulin resistance associated with increased ectopic fat and oxidative stress (Mei et al., 2014). Humans consuming diets high in fat (>35% of total kCal intake), generally report increased saturated fat intakes, and are at risk for health benefits due to overconsumption of calories (Ehrman, 2010). No significant relationships were found between apelin and the reported total kcal intake or macronutrient percentages of total kcal intake (%CHO, %Protein, % Fat).

Antioxidant Consumption

The subjects' dietary intake was analyzed via 3-day food logs and their micronutrient consumption was evaluated using the USDA food nutrient database. This provided the subjects' Vitamin A, C and E intake based on the reported food consumption. The USDA Nutrient Data Laboratory reported vitamin A in retinol activity equivalents (RAE - mcg) and vitamins C, E and zinc in milligrams (mg). It was hypothesized that individuals with low antioxidant consumption will have increased plasma apelin concentrations. However, no subjects reported consuming low levels of antioxidants in their diets.

Vitamins A, C, and E were analyzed individually, and then pooled to explore possible relationships between these micronutrients and plasma apelin concentration at rest. It appears that vitamins A, C, and E may influence plasma apelin concentrations in different ways. Vitamins A and C intake indicate a slight negative correlation, however this relationship is weak (Vitamin A: P=0.592; Vitamin C: P=0.496). Conversely, Vitamin E suggests a significant positive correlation (r=0.658, P=0.020) One subject

reported consuming >200% of the recommended daily intake of the antioxidants, this subject may have skewed the results.

In order to calculate total antioxidant consumption the vitamin percentage of summed vitamins A, C, and E compared to the RDA, giving a total percentage of recommended antioxidants consumed. Once pooled, the percentage of RDA consumed and plasma apelin did not have a significant relationship, but ($r=0.195$, $P=0.544$). From this data, the hypothesis stating that increased antioxidant intake would be correlated with lower plasma apelin concentrations cannot be supported or fully rejected. There were not enough individuals outside of the RDA range to analyze high versus low consumption.

Zinc Consumption

In addition to vitamins A, C, and E, reported zinc consumption was also analyzed using the USDA nutrient database to establish an average daily consumption for each subject. It was proposed that those subjects consuming a higher zinc intake would have decreased apelin concentrations at rest. This hypothesis cannot be accepted. The data collected suggests that there is no significant relationship between average daily zinc consumption (mg) and plasma apelin concentrations at rest.

Apelin

As shown (see table 13), there have been wide ranges of plasma apelin concentrations reported by various research groups. Our values of 0.511±0.333 are close to the average (0.635 ng/mL) of the previously reported apelin concentrations in healthy human controls. At first it was thought that the difference in reported apelin

concentrations was due to the year the values were reported in which studies by Papadopoulos (2013), Mesmin (2010), and Castan-Laurell (2008) all seemed similar. In addition, it seemed that several of the more recent studies have reported significantly higher apelin scores including Alexiadou (2012) and Kadoglou (2012), using kits purchased from the same company, but shipped from a different location (Belmont, CA and Strausburg, France) suggesting there may have been a difference in assay kits used between research groups. Therefore comparisons are difficult within the literature, even when using similar Phoenix Pharmaceutical apelin-12 kits, as the facility it originates from seems to be a factor.

Future Directions

It is suggested that future studies should include a larger cohort of subjects with a wider range of diets as well as a larger range for each of the reported variables. To further look at diet, future studies should control for diet in interventional as well as observational studies. It is not suggested that the high antioxidant intakes should be looked at further as there have been studies reporting negative side effects to high antioxidant supplementation. It was difficult to analyze antioxidant intake versus apelin concentration as we only had 1 subject who exhibited high antioxidant intake (>200% RDA) and we had no subjects reporting extremely low antioxidant intake. Future studies should include larger cohorts including a wider range for BMI due to inconsistency between studies, where several found no relationship between BMI and apelin(Chong, Gardner, Morton, Ashley, & McDonagh, 2006; Erdem, Dogru, Tasci, Sonmez, & Tapan, 2008; Soriguer et al., 2009; Tasci et al., 2007). In terms of body fat distribution, more

sophisticated forms of measurement should be used such as MRI and/or DEXA would be useful tools to analyze the role of body fat distribution on plasma apelin concentrations.

It is suggested that this study should be done with a larger cohort and with subjects of a wider range of calorie and nutrient intakes to determine if the addition of a high fat diet would influence resting plasma apelin concentration. It will also be important to indicate the source of fats and type of fats (MUFA, PUFA, SFA) in future studies as it is suggested that the types of fat consumed have more of an effect on increased metabolic disturbances than total fat intake (Alhazmi et al., 2012; Peairs et al., 2011). Studies have shown that postprandial oxidative stress, particularly in AT is influenced by the various types of fats ingested (Meza-Miranda et al., 2014). Therefore, not only is the overall amount of fat consumed a concern, but the type of fats consumed may also influence oxidative stress. It is important to note that there are variations in the different types of fat consumed such as saturated fatty acids (SFAs), polyunsaturated fatty acids (PUFAs) and its subtypes, and monounsaturated fatty acids (MUFAs). Studies have investigated the protective effects of MUFAs and PUFAs versus SFAs in terms of oxidative stress and IR, but it remains unclear as to how ingestion of these fats may influence apelin levels at rest or in response to exercise/glucose stimuli.

Conclusion

In conclusion, this study includes a small cohort of subjects all had stable resting plasma apelin levels across visits and it does not appear that their dietary factors influenced their apelin levels. In contrast, %BF, BMI and SAD suggest that these factors

are significantly related to resting plasma apelin whereas WC approached significance. Dietary intakes showed no significant effect on apelin.

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APPENDIX A

HEALTH HISTORY, DRUG USAGE, AND
FITNESS ACTIVITY QUETIONNAIRE

Subject: _____
ID: _____

Health History

Blood Pressure: SBP/DBP: _____

Do you have any musculoskeletal illnesses or injuries that would restrict your participation in either a maximal or submaximal exercise bout (as performed on a treadmill or with use of free weights while performing a squatting motion)?

YES NO

If yes, please describe._____

_____

Have you ever been diagnosed with cardiovascular disorders (heart problems, high blood pressure, high cholesterol, etc.)? YES NO

If yes, please describe._____

_____

Have you ever been diagnosed with metabolic disorders (diabetes, etc.)?

YES NO

If yes, please describe._____

_____

Could you currently be pregnant? YES NO N/A

Please list any major illnesses, hospitalizations, or surgical procedures within the last two years.

_____

_____

Drug/Supplement Usage

Are you a *current* smoker/Tobacco user? YES NO

Have you every smoked/tobacco use in the past? YES NO

If yes, please describe history._____

_____

Are you currently taking any medication(s)? YES NO

If yes, please list name of medication(s), reason for and length of administration.

_____

_____

Are you currently taking any nutritional/vitamin supplements?
YES NO

If yes, please list name of supplement, reason for and length of administration.

_____

Are you taking energy bars or drinks on a routine basis? YES NO

Fitness Activity

Please describe your current participation in the following types of exercise:

1. Aerobic (aerobic classes, walking, jogging, stair climbing, hiking, cycling, running swimming etc.) If more than one activity please list separately.

Frequency (# of days per week): _____
Duration (time spent per session): _____minutes
Intensity (difficulty level): light somewhat hard hard very hard

How long have you been participating in the aerobic activity described above?
Less than three months 3-6 months 6-12 months greater than 12 months

Additional comments:_____

_____

_____

2. Anaerobic (weight training, sprinting, boxing, martial arts, interval training, etc.)

Frequency (# of days per week): _____
Duration (time spent per session): _____minutes
Intensity (difficulty level): light somewhat hard hard very hard

How long have you been participating in anaerobic activity as described above?
Less than three months 3-6 months 6-12 months greater than 12 months

Additional comments:_____

_____

3. Organized or Recreational sports (basketball, soccer, lacrosse, etc.)

Type of sport(s):_____

Frequency (# of days per week): _____
Duration (time spent per session): _____minutes
Intensity (difficulty level): light somewhat hard hard very hard

How long have you been participating in sports activity as described above?
Less than three months 3-6 months 6-12 months greater than 12 months

Additional comments:_____

4. Other activities

Type of activities (Thai chi, yoga, stretching activities,
Classification (to be assessed by researchers—leave blank)
Need to confirm aerobic capacity by maximal oxygen uptake test.

Trained Untrained

APPENDIX B

AHA/ACSM HEALTH/FITNESS FACILITY PRE-PARTICIPATION SCREENING QUESTIONNAIRE

Assess your health status by marking all true statements

History:

You have had:

_____ a heart attack
_____ heart surgery
_____ cardiac catheterization coronary
_____ angioplasty (PTCA)
_____ Pacemaker/implantable cardiac defibrillator
_____ rhythm disturbance
_____ heart valve disease
_____ heart failure
_____ heart transplantation
_____ congenital heart disease

Symptoms:

_____ You experience chest discomfort with exertion
_____ You experience unreasonable breathlessness
_____You experience dizziness, fainting, or blackouts
_____ You take heart medications

Other health issues:

_____ You have diabetes
_____ You have asthma or other lung disease
_____ You have burning or cramping sensation in your lower legs when walking short distances
_____ You have musculoskeletal problems that limit your physical activity
_____ You have concerns about the safety of exercise
_____ You take prescription medication(s)
_____ You are pregnant

If you marked any of these statements in this section, consult you physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

Cardiovascular risk factor :

_____ You are a man older than 45 years
_____ You are a woman older than 55 years, have had a hysterectomy, or are postmenopausal
_____ You smoke, or quit smoking within the previous 6 month
_____ Your blood pressure is >140/90 mm Hg
_____ You do not know your blood pressure
_____ You take blood pressure medication
_____ Your blood cholesterol level is > 200 mg/dl
_____ You do not know your cholesterol level
_____ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)
_____ You are physically inactive (i.e., you get <30 minutes of physical activity on at least 3 per week)
_____ You are >20 pounds overweight

If you marked two or more of the statements in this section you should consult your physician or other appropriate health care provider before engaging in exercise. You will benefit from using our facility with a professionally qualified exercise staff to guide your exercise program.

_____ None of the above

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility including our facility that meets your exercise program needs.

Modified from American College of Sports Medicine and American Heart Association. ACSM/AHA Joint Position Statement: Recommendations for cardiovascular screening, staffing, and emergency policies at health/fitness facilities. Medicine and Science in Sports and Exercise 1998: 1018

APPENDIX C

SKINFOLD MEASUREMENT DATA FORM

Chest _____ _____
Average_____

Triceps _____ _____ Average_____

Axilla _____ _____
Average_____

Subscapular _____ _____ Average_____

Abdominal _____ _____ Average_____

Suprailium _____ _____ Average_____

Thigh _____ _____ Average_____

Total ∑7 = _____mm Total ∑3 = _____mm

Males:

Db (∑3) = 1.10938 - (0.0008267 x ∑3) + (0.0000016 x$(\sum 3)^2$) - (0.0002574 x age)

Db (∑7) = 1.112 - (0.00043499 x ∑7) + (0.00000055 x$(\sum 7)^2$) -(0.00028826 x age)

Women:

Db (∑3) = 1.0994921 - (0.0009929 x ∑3) + (0.0000023 x$(\sum 3)^2$) - (0.0001392 x age)

Db (∑7) = 1.097 - (0.00046971 x ∑7) + (0.00000056 x$(\sum 7)^2$) - (0.00012828 x age)

% Body Fat = {(4.95/ D_b) - 4.50} x 100 (Siri, 1961).

% Body Fat = {(4.57/ D_b) - 4.142} x 100 (Brozek et al., 1963).

Siri (1961)
% Body Fat_____(∑3)
% Body Fat_____(∑7)

Brozek (1963)
% Body Fat_____(∑3)
% Body Fat_____(∑7)

APPENDIX D

VO2MAX TEST DATA FORM

Date:_____
Subject:_____
ID:_____
Gender:_____
Age:_____
Height:_____
Weight:_____
Resting HR:_____
Resting BP:_____
Relative Humidity:_____
Waist to Hip Ratio _____
Atmospheric Pressure:_____
Sagittal girth _____
Room Temperature:_____

Time (min)	Workload speed/grade	HR (bpm)	RPE	VO_2 (ml/kg/min)	VCO_2 (ml/kg/min)	R	V_E (L/min)	Comments
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								

Blood taken pre _____ post _____ 1hr post ____ 24hr _____
Glucose concentration pre _____ post _____ 1hr post ____ 24hr _____
Hct pre _____ post _____ 1hr post ____ 24hr _____
Diet papers brought in and discussed _____ Returned a copy ____

APPENDIX E

FOOD DIARY DATA FORM

Please list **ALL food and drink** consumed during the three days before each experimental day. Use a separate sheet for each day and refer to the following examples for ideas on how specific you should be. Please include all added antioxidants (Vit C, E, A, etc.).

7:00 a.m.	Eggs (fried) wheat bread jelly (grape) orange juice (conc.)	2 whole (jumbo) 4 whites (jumbo) 3 slices 3 tsp. 2 cups	180 75 300 ? 200
11:00 a.m.	Rice (instant/white) baked chicken (boneless/white meat) olive oil hamburger on bun (with ketchup, mustard, mayo, lettuce, tomato and onions)	1 cup (dry) 8 oz. 2 tbs. 4oz	 300 300 240 360

Time	*Food source*	*Quantity (oz./cups)*	*Calories (if known)*

Name: Day: Date:

APPENDIX F

UNCG CONSENT TO ACT AS A HUMAN PARTICIPANT

Project Title: Acute effects of exercise on apelin, glucose, insulin and insulin sensitivity in apparently healthy individuals

Principal Investigator and Faculty Advisor (if applicable): Justin Waller (PI) and Dr. Allan Goldfarb

Participant's Name: _____

What is the study about?

This is a research project. Your participation is voluntary. Your fat cells send signals to other tissues and one of these is known as apelin. The amount of fat you have influences blood apelin level and alters glucose balance and insulin sensitivity. Exercise serves as an excellent model to alter blood glucose levels and enhance insulin sensitivity. Apelin's role on insulin sensitivity and glucose balance in response to acute exercise has not been investigated. We intend to determine how moderate and high intensity acute aerobic exercise influence blood apelin level, along with blood insulin and blood glucose. It is the purpose of this study to see if apelin levels in the blood change to either a high intensity aerobic exercise known to enhance blood glucose levels compared to a moderate intensity exercise known to maintain blood glucose levels.

Why are you asking me?

You are being asked to participate in this study because little research has been done regarding apelin and its effects on glucose homeostasis and insulin sensitivity in apparently healthy human subjects. You also fit our criteria of being between the ages of 18-35. Prior to your participation, you will complete an American Heart Association/American College of Sports Medicine health fitness facility pre-participation screening questionnaire and a physical activity form. This is done in order to screen out individuals who may have musculoskeletal, cardiovascular or metabolic disorders that may interfere with the testing procedures or the measurements obtained, or women who may be pregnant or breast feeding. Only volunteers that do not use tobacco products (minimum 6 months) may be enrolled. Also, if you have been on any antioxidant supplement you must discontinue use for at least two weeks prior to being accepted as a subject. If you are taking any medication or drugs that may influence glucose, insulin or your metabolism/inflammation you will not be allowed to participate in this study. If your resting fasting blood glucose is above 100 mg/dl you will be excluded. If your resting blood pressure is elevated (140/90 mmHg) you will also be excluded from participating.

What will you ask me to do if I agree to be in the study?

You will be asked to participate in this study through six (6) visits to our laboratory. Two of these visits will involve exercise and blood sampling. Another visit will involve ingestion of a glucose gel and blood sampling and three visits will only involve blood sampling.

VISIT 1: You will be pre-screened for the information provided in the preceding section of this consent form. Screening will occur on the morning of your first visit. You will also bring a 3-day food record on your first visit listing amounts of food and drink. **The time for this screening should be no longer than 15 minutes**.

After screening you will have your height, weight, blood pressure and body fat percentage (using a skinfold caliper at seven sites) determined. A resting blood sample from an arm vein will then be obtained (approximately 3 teaspoons, or 14 mls); you may feel slight discomfort or pain with the needle stick. You will then perform a graded treadmill run to determine your aerobic capacity (VO_2max). The grade and speed of the treadmill will be increased gradually over time as you breathe through a mouthpiece connected to a tube to enable expired air samples to be measured for oxygen content. The test will continue until your aerobic capacity is reached or you indicate you can no longer maintain the pace or want to stop; you may feel some discomfort/fatigue during this test. There are bars on either side of the treadmill to grasp if needed. You will then be given a one-minute cool down/walk, after which another blood sample (3 teaspoons) will be obtained. Another resting, fasted blood sample (3 teaspoons) will be obtained 60 minutes after exercise. You will be provided water throughout this visit. You will press on the needle stick area for 2-5 minutes to avoid bruising after each of these blood samples is taken. **The total time for this part of the visit will be no more than 1 hour and 45 minutes.**

VISIT 2: After 24 hours you will visit the laboratory to have a 24-hr post-exercise blood sample (3 teaspoons) obtained; you must come in after an overnight (8-10 hrs) fast. The procedures for this blood draw will follow the same as the previous blood draws. **The resting, fasted 24-hour blood sample will take less than 15 minutes to collect.**

VISIT 3: You will return to the lab at least 7 days after the initial graded run in the morning (same time and condition) to perform either a 30-minute run at a moderate intensity (75% of your aerobic capacity) or ingest a glucose gel about 50 grams). Your weight, blood pressure and resting heart rate will be determined as before. You will have blood taken at rest (3 teaspoons at each time point), immediately post-exercise (or gel consumption) and 60 minutes post-exercise (or gel consumption). Your heart rate and oxygen consumption will be monitored every 5 minutes during the run or after the ingestion of the gel. You will also give us feedback as to the difficulty of the exercise. You will be given water throughout this visit. You will also bring a 3-day food record prior to the third visit. **The total time for this visit will be no more than 2 hours.**

VISIT 4: After 24 hours you will visit the laboratory to have a 24-hr post-exercise blood sample (3 teaspoons) obtained; you must come in after an overnight (8-10 hrs) fast. The procedures for this blood draw will follow the same as the previous blood draws. **The resting, fasted 24-hour blood sample will take less than 15 minutes to collect.**

VISIT 5: You will return to the lab at least 7 days after visit 3 in the morning (same time and condition) to perform the treatment you did not complete in visit 3. Your weight, blood pressure and resting heart rate will be determined as before. You will have blood taken at rest (3 teaspoons at each time point)), immediately post-exercise (or gel consumption) and 60 minutes post-exercise (or gel consumption). Your heart rate and oxygen consumption will be monitored every 5 minutes during the run or after the ingestion of the gel. You will also give us feedback as to the difficulty of the exercise. You will be given water throughout this visit. You will also bring a 3-day food record prior to the third visit. **The total time for this visit will be no more than 2 hours.**

VISIT 6: After 24 hours you will visit the laboratory to have a 24-hr post-exercise blood sample (3 teaspoons) obtained; you must come in after an overnight (8-10 hrs) fast. The procedures for this blood draw will follow the same as the previous blood draws. **The resting, fasted 24-hour blood sample will take less than 15 minutes to collect.**

This will conclude your study participation. **Total participation time will be about 6.75 hours.**

All information concerning your records will be kept confidential and you will not be identified in any presentation or published work.

In addition, all blood samples obtained will be kept for possible future analyses and research, though you will not be identified in any way.

You may contact the PI, Justin Waller, at jdwaller@uncg.edu or (336) 613-6899 or Dr. Allan Goldfarb at ahgoldfa@uncg.edu or (336) 334-3029 with any questions concerning the nature of your participation and your consent to participate in this research project.

What are the risks to me?

The specific risks associated with participation in this research project are listed below.

You may feel tired and out of breath after the maximum aerobic capacity test which is normal. There is a very small risk of death as a result of this test (< 0.01%) and this is for all age groups and includes individuals that might have health problems. Since you are young and do not have any known health problems the risks associated with this test are reduced. You may let the researcher know if you are experiencing discomfort during the maximum aerobic capacity test, at which time the test will be discontinued. You may feel tired after the 30 minute run, which is normal.

It is possible that you may feel some symptoms of low blood sugar during either of the exercise sessions. Some of these symptoms are:

- Shakiness or hand tremors
- Ringing in the ears
- Chills or clamminess
- Lightheadedness or dizziness
- Irritability
- Nausea

In the event that you feel these symptoms, you should immediately inform the researcher so that he/she may discontinue the test. To reduce the risk to you, a safety 'spotter' will be in place at all times during the testing period to assist you.

Sterile techniques will be utilized to take the blood and there may be a small sensation of pain associated with taking blood. There may be some slight bruising associated with the blood sampling; placing direct pressure over the area where the blood was taken will minimize this. You must realize that infection can occur with any type of blood drawing but following sterile techniques will reduce this risk.

If you have questions, want more information or have suggestions, please contact: Justin Waller at jdwaller@uncg.edu or (336) 613-6899 or Dr. Allan Goldfarb at (336) 334-3029 or ahgoldfa@uncg.edu.

If you have any concerns about your rights, how you are being treated, concerns or complaints about this project or benefits or risks associated with being in this study, please contact the Office of Research Integrity at UNCG toll-free at (855)-251-2351.

What happens if I am injured during this study?

UNCG is not able to offer financial compensation nor to absorb the costs of medical treatment should you be inured as a result of participating in this research study. However, we will provide a referral to your primary care physician.

Are there any benefits to society as a result of me taking part in this research?

This research project may advance the exercise science literature. Apelin has recently been reported to influence insulin sensitivity and glucose regulation, which carries important implications for the treatment of diabetes. Findings from this research project may advance future research in this area.

Are there any benefits to me for taking part in this research study?

Through your participation in this study, you will learn of your maximal aerobic capacity, percent body fat and blood pressure. Additionally, you will be able to receive information on how you responded to the exercise sessions. You can ask for the blood glucose data to see if you maintained blood glucose balance on the 30 minute run. This information may be important in improving your overall health, and may serve as baseline data related to these measures. Otherwise, the information gathered in this study may not directly benefit you; however, it may advance the exercise science literature.

Will I get paid for being in the study? Will it cost me anything?

There are no costs to you or payments made to you for participating in this study.

How will you keep my information confidential?

All information concerning your records and obtained data will be kept confidential and you will not be identified in any presentation or published work. You will receive a study number, which will not be linked to your name. All records and data related to you, specifically, will be de-identified and coded and will be safely kept in a locked file cabinet. The master list for this study will also be codified with no identifying information. All study data will be destroyed, via document shredding and file erasure, upon publishing of the research data. This consent form will be kept for three years as required by federal law, after which time they will be destroyed to protect your identity. Blood samples will be properly disposed of after manuscripts have been published in accordance with biological hazard codes. All information obtained in this study is strictly confidential unless disclosure is required by law.

What if I want to leave the study?

You have the right to refuse to participate or to withdraw at any time, without penalty. If you do withdraw, it will not affect you in any way. If you choose to withdraw, you may request that any of your data which has been collected be destroyed unless it is in a de-identifiable state.

What about new information/changes in the study?

If significant new information relating to the study becomes available which may relate to your willingness to continue to participate, this information will be provided to you.

Voluntary Consent by Participant:

By signing this consent form/completing this survey/activity (used for an IRB-approved waiver of signature) you are agreeing that you read, or it has been read to you, and you fully understand the contents of this document and are openly willing consent to take part in this study. All of your questions concerning this study have been answered. By signing this form, you are agreeing that you are 18 years of age or older and are agreeing to participate, or have the individual specified above as a participant participate, in this study described to you by either Justin Waller or Dr. Allan Goldfarb.

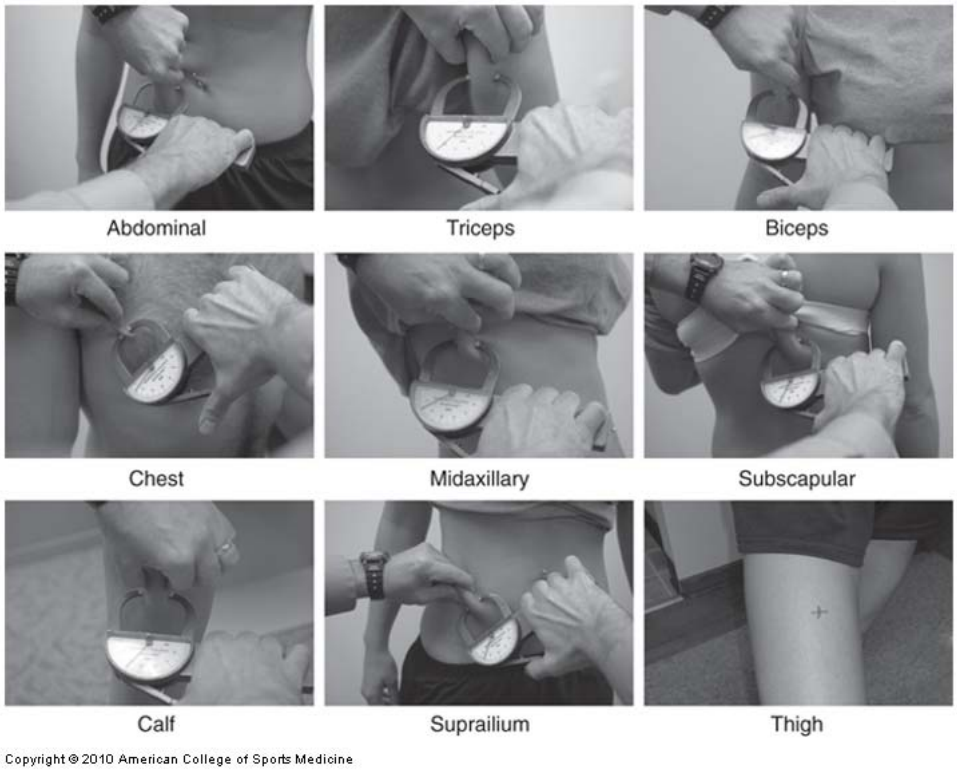
Signature: _____ Date: _____

APPENDIX G

ACSM SKINFOLD MEASUREMENT PROCEDURES

ACMS Resource Manual for Exercise Testing and Prescription (p.270)

Figure 17-1. Skinfold Measures



Skinfold Site	Procedure	
Abdominal	Vertical Fold	2 cm to the right side of the umbilicus
Triceps	Vertical Fold	On the posterior midline of the upper arm, halfway between the acromion and olecranon processes, with the arm held freely to the side of the body
Chest	Diagonal Fold	1/2 the distance between the anterior axillary line and the nipple (men); or 1/3 the distance between the anterior axillary line and the nipple (women)
Midaxillary	Vertical Fold	On the midaxillary line at the level of the xiphoid process of the sternum
Subscapular	Diagonal Fold (45° angle)	1 to 2 cm below the inferior angle of the scapula
Suprailiac	Diagonal Fold	In line with the natural angle of the iliac crest taken in the anterior axillary line immediately superior to the iliac crest
Thigh	Vertical Fold	On the anterior midline of the thigh midway between the proximal border of the patella and the inguinal crease (hip)

ACSM Resource Manual for Guidelines for Exercise Testing and Prescription pg. 270

APPENDIX H

RAW DATA

Subject Characteristics

Subject	*Age*	*Gender*	*Weight (kg)*	*Height (cm)*	*BMI (kg/m^2)*	*Siri BF%*	*Brozek BF%*	*SAD (cm)*	*WC (cm)*	*Hip (cm)*	*WHR*
1	27	Male	66.1	172	22.3	6.69	7.43	18.25	78	81	0.963
2	22	Female	59.15	155.5	24.5	23.42	22.89	19.1	72.5	91.2	0.79
3	29	Female	76.3	165.5	27.8	32.33	31.11	24.8	83	104.8	0.792
4	23	Female	65.55	177	20.9	16.5	16.5	18.25	69	85	0.812
5		Male	79.85	182	24.2	10.68	11.11	21	81.5	100.5	0.811
6	20	Male	68.05	177	21.7	7.67	8.34	18	71	91.5	0.776
7	21	Male	105.3	182	31.9	23.36	22.82	27.1	97.5	115	0.848
8	21	Male	67.85	171	23.2	11.84	12.19	19	76.5	95	0.805
9	21	Female	56.05	167	20.1	18.21	18.07	17.5	72.5	92	0.788
10	21	Male	55.65	168	19.7	4.25	5.18	16.5	67.5	85	0.794
11	26	Male	64.9	164	24.1	13.64	13.84	20	80.25	94.5	0.849
12	21	Female	63.6	169.5	22.1	15.73	15.78	18.4	70	100	0.7
13	22	Male	60.05	177	19.2	7.87	8.53	17.6	68.5	89	0.769
14	21	Female	53.1	165	19.5	18.68	18.5	15.4	63.5	92.4	0.687
15	20	Male	72.75	177	23.2	6.08	5.22	19.75	77	97.5	0.789

Body Composition Measurements

Subject	*Age*	*Gender*	*Chest (mm)*	*Tricep (mm)*	*Axilla (mm)*	*Sub-scapular (mm)*	*Abdominal (mm)*	*Supra-ilium (mm)*	*Thigh (mm)*	*Body Density*
1	27	Male	3.85	4.2	5.85	8.85	11.2	10.15	5.75	1.08
2	22	Female	17.35	20.05	13.3	16.35	18.2	18.15	17.5	1.05
3	29	Female	29.05	27.65	27.95	16.25	30.4	21	30	1.03
4	23	Female	7.25	7.75	15.5	6	15.25	7	18.5	1.06
5		Male	9.1	7.3	9.15	13.2	19.3	9.2	9.9	1.07
6	20	Male	6.35	9.65	5.9	9	9.75	7.5	13.35	1.08
7	21	Male	21.75	27.65	24.1	24.3	33.15	25.2	23.05	1.05
8	21	Male	12.5	13.6	9.4	13.95	13.7	8.15	17.1	1.07
9	21	Female	8.25	14.5	8.75	8.5	15.5	9.75	23	1.06
10	21	Male	4.9	5	4.9	6	6.1	3.95	8.5	1.09
11	26	Male	9.05	9.9	12.35	15.3	20.2	9.9	19.8	1.07
12	21	Female	5.55	11.2	7.5	7.95	10.5	7.95	23.1	1.06
13	22	Male	5	5.6	6.1	10.65	14.3	6.35	13.25	1.08
14	21	Female	12.35	12.4	11.95	10.2	16	8.45	19.8	1.06
15	20	Male	5.2	6.3	5.2	8	6.9	5.1	9.35	1.09

Reported Total kCal Consumed

Subject		*Total kcal 1*	*Total kcal 2*	*Total kcal 3*	*Avg total kCal*
1	male	1323	1270.5	1393.75	1329.08
2	female	1631.43	676.33	1583.25	1297
3	female	2827.33	2349.33	2247	2474.56
4	female	1617.67	2435.67	2287.5	2113.61
6	male	2271.67	2769.75	3045	2695.47
7	male	3471.75	3023	2769.5	3088.08
8	male	3000.75	3217	3636.25	3284.67
11	male	1038.75	903.5	1097.25	1013.17
12	female	1584.75	1436.33	1863.25	1628.11
13	male	1612	2235.25	2544.25	2130.5
14	female	1446.75	1450.25	1009	1302
15	male	3456.5	3969.33	3321	3582.28

Reported CHO intakes

Subject		*CHO 1 (g)*	*CHO2 (g)*	*CHO 3 (g)*	*Avg CHO (g)*
1	male	178.23	210.86	185.55	191.55
2	female	212.18	100.07	166.83	159.69
3	female	331.34	289.16	256.78	292.43
4	female	86.03	358.16	219.88	221.35
6	male	252.59	296.82	382.69	310.69
7	male	381.95	414.41	372.88	389.75
8	male	366.27	405.21	512.68	428.05
11	male	76.22	99.69	97.28	91.06
12	female	216.38	193	233.75	214.38
13	male	228.34	285.34	334.69	282.79
14	female	129.56	139.08	153.57	140.74
15	male	438.46	454.33	378.23	423.67
Subject		CHO 1 (kCal)	CHO 2 (kCal)	CHO 3 (kCal)	Avg CHO (kCal)
1	male	712.93	843.45	742.2	766.19
2	female	848.7	400.27	667.32	638.76
3	female	1325.36	1156.64	1027.11	1169.70
4	female	344.12	1432.63	879.5	885.42
6	male	1010.35	1187.27	1530.77	1242.79
7	male	1527.79	1657.65	1491.51	1558.98
8	male	1465.06	1620.85	2050.7	1712.20
11	male	304.88	398.74	389.12	364.25
12	female	865.5	772.01	935.01	857.51
13	male	913.37	1141.34	1338.77	1131.16
14	female	518.22	556.33	614.28	562.94
15	male	1753.84	1817.33	1512.9	1694.69

Reported Protein Intakes

Subject		*Protein 1 (g)*	*Protein 2 (g)*	*Protein 3 (g)*	*Avg Pro (g)*
1	male	80.91	61.90	69.15	70.65
2	female	81.20	21.72	64.55	55.82
3	female	114.36	96.48	98.54	103.12
4	female	122.62	81.79	128.35	110.92
6	male	157.86	184.45	195.91	179.41
7	male	98.97	72.22	88.71	86.63
8	male	143.37	153.14	159.89	152.13
11	male	77.65	56.45	77.75	70.62
12	female	76.25	50.56	69.35	65.39
13	male	58.38	77.46	90.89	75.58
14	female	80.78	91.01	43.90	71.89
15	male	142.8	157.38	146.57	148.81

Subject		*Pro 1 (kCal)*	*Pro2 (kCal)*	*Pro 3 (kCal)*	*Avg Pro (kCal)*
1	male	323.65	247.58	276.59	282.61
2	female	324.79	86.87	258.21	223.29
3	female	457.43	385.91	394.15	412.49
4	female	490.48	327.16	513.4	443.68
6	male	631.43	737.79	783.65	717.62
7	male	395.86	288.88	354.82	346.52
8	male	573.48	612.54	639.54	608.52
11	male	310.59	225.81	310.98	282.46
12	female	305.02	202.23	277.38	261.54
13	male	233.51	309.85	363.54	302.3
14	female	323.13	364.02	175.58	287.58
15	male	569.9	629.53	586.27	595.23

Reported Fat Intakes

Subject		*Fat 1 (g)*	*Fat 2 (g)*	*Fat 3 (g)*	*Avg Fat (g)*
1	male	36.70	32.86	45.88	38.48
2	female	56.05	21.52	75.56	51.04
3	female	101.24	95.11	115.84	104.06
4	female	97.12	112.59	104.73	104.82
6	male	75.00	87.92	85.72	82.88
7	male	157.29	123.58	107.915	129.60
8	male	98.69	102.25	115.65	105.53
11	male	47.023	31.64	40.56	39.74
12	female	43.67	57.21	61.02	53.96
13	male	53.70	60.90	83.79	66.13
14	female	52.33	43.35	25.92	40.53
15	male	156.85	181.54	149.55	162.63

Subject		*Fat 1 (kCal)*	*Fat 2 (kCal)*	*Fat 3 (kCal)*	*Avg Fat (kCal)*
1	male	330.26	295.76	412.92	346.31
2	female	504.47	193.65	680.06	459.40
3	female	911.16	856.02	1042.56	936.58
4	female	874.11	1013.34	942.57	943.34
6	male	675.03	791.30	771.45	745.93
7	male	1415.66	1112.22	971.24	1166.37
8	male	888.26	920.21	1040.85	949.77
11	male	423.20	284.74	365.04	357.66
12	female	392.99	514.86	549.14	485.66
13	male	483.32	548.12	754.09	595.18
14	female	470.97	390.17	233.26	364.8
15	male	1411.67	1633.83	1345.63	1463.71

Reported SFA Intakes

Subject		*SFA 1*	*SFA 2*	*SFA 3*	*Avg SFA (g)*
1	male	12.26	9.48	15.88	12.54
2	female	17.59	5.96	18.77	14.11
3	female	18.54	24.06	28.10	23.57
4	female	22.85	34.31	28.58	28.58
6	male	29.10	27.88	29.58	28.85
7	male	45.91	40.82	37.86	41.53
8	male	24.40	26.21	27.99	26.20
11	male	18.61	12.59	14.28	15.16
12	female	13.27	16.03	20.01	16.44
13	male	22.31	29.09	32.71	28.04
14	female	23.39	14.03	8.15	15.19
15	male	45.48	60.92	51.93	52.78

Reported MUFA Intakes

Subject		*MUFA 1*	*MUFA 2*	*MUFA 3*	*Avg MUFA (g)*
1	male	47.04	50.55	44.16	47.25
2	female	17.72	7.73	30.35	18.60
3	female	26.27	30.62	52.29	36.39
4	female	27.38	31.07	31.87	30.11
6	male	16.07	37.04	26.82	26.64
7	male	40.42	48.51	35.78	41.57
8	male	38.43	32.06	44.84	38.45
11	male	16.89	11.11	15.69	14.56
12	female	14.54	16.08	19.20	16.61
13	male	15.53	12.82	24.44	17.60
14	female	5.09	14.65	8.39	9.377
15	male	51.07	54.39	48.57	51.34

Reported PUFA Intakes

Subject		*PUFA 1*	*PUFA 2*	*PUFA 3*	*Avg PUFA (g)*
1	male	7.98	7.31	7.15	7.48
2	female	15.63	6.70	18.43	13.58
3	female	18.93	14.37	32.68	21.99
4	female	88.39	34.05	42.46	54.96
6	male	10.51	21.29	16.05	15.95
7	male	45.56	23.32	19.22	29.36
8	male	25.12	22.99	32.75	26.95
11	male	4.45	5.15	7.91	5.84
12	female	11.39	16.69	12.28	13.46
13	male	10.81	9.74	18.59	13.05
14	female	3.53	6.34	5.06	4.98
15	male	27.39	23.74	31.06	27.39

Reported Vitamin A Intakes

Subject		*Vit A1 (mg)*	*Vit A2 (mg)*	*Vit A3 (mg)*	*AvgVit A (mg)*	*Vit A% of RDA*
1	male	1.43	2.66	2.05	2.05	1.40
2	female	0.40	0.13	0.25	0.26	0.23
3	female	0.27	0.36	0.81	0.48	0.44
4	female	0.80	0.30	1.71	0.94	0.72
6	male	1.36	1.38	3.07	1.94	0.41
7	male	1.15	1.37	1.01	1.18	0.47
8	male	1.93	1.94	1.76	1.88	0.50
11	male	1.04	0.63	0.41	0.69	0.46
12	female	3.30	3.96	1.74	3.00	0.63
13	male	0.61	0.43	2.87	1.30	0.48
14	female	0.36	0.44	0.40	0.399	0.61
15	male	2.93	1.95	2.68	2.52	0.541

Reported Vitamin C Intakes

Subject		*Vit C1 (mg)*	*Vit C2 (mg)*	*Vit C3 (mg)*	*AvgVit C (mg)*	*Vit C % RDA*
1	male	256.1	463.73	490.05	403.29	5.45
2	female	110.40	14.2	59.55	61.38	0.79
3	female	54.35	132.9	6.09	64.45	0.081
4	female	16.5	58.16	135.45	70.04	1.81
6	male	189.38	66.61	50.36	102.11	0.56
7	male	70.01	80.9	85.98	78.96	0.96
8	male	145.33	77.43	220.47	147.74	2.45
11	male	45.63	80.6	84.05	70.09	0.93
12	female	314.51	165.87	64.21	181.53	0.86
13	male	19.75	76.73	58.78	51.75	0.65
14	female	208.48	24.88	32.31	88.55	0.431
15	male	168.98	113.1	153.12	145.08	1.70

Reported Vitamin E Intakes

Subject		*Vit E1 (mg)*	*Vit E2 (mg)*	*Vit E3 (mg)*	*AvgVit E (mg)*	*Vit E % RDA*
1	male	9.99	12.31	10.62	10.97	0.707
2	female	12.02	12.90	20.64	15.18	1.376
3	female	4.97	8.61	14.33	9.30	0.955
4	female	8.13	16.10	68.14	30.79	4.542
6	male	6.31	26.27	42.10	24.89	2.806
7	male	18.22	48.94	22.83	30.00	1.522
8	male	58.75	45.99	35.20	46.64	2.35
11	male	3.89	5.14	3.12	4.05	0.208
12	female	10.96	11.97	10.69	11.21	0.713
13	male	4.42	5.39	6.49	5.43	0.432
14	female	4.81	5.27	3.33	4.47	0.222
15	male	12.75	13.51	13.63	13.30	0.9084

Reported Zinc Intakes

Subject		*Zinc 1 (mg)*	*Zinc 2 (mg)*	*Zinc 3 (mg)*	*Avg Zinc (mg)*	*Zinc % RDA*
1	male	12.27	7.92	12.34	10.84	1.12
2	female	12.08	3.53	7.47	7.69	0.93
3	female	7.59	9.33	8.83	8.58	1.10
4	female	10.81	10.39	9.29	10.16	1.16
6	male	13.11	19.81	21.21	18.04	1.93
7	male	9.19	9.37	8.34	8.97	0.76
8	male	20.35	26.02	28.49	24.95	2.59
11	male	14.52	6.10	8.75	9.79	0.79
12	female	8.58	6.43	7.64	7.55	0.96
13	male	6.17	6.12	9.49	7.26	0.86
14	female	2.42	11.47	6.62	6.83	0.83
15	male	20.61	35.18	20.83	25.54	1.89

Plasma Apelin Concentrations at Rest

Subject		*Apelin A*	*Apelin B*	*Apelin C*	*Average Apelin*
1	male	0.393	0.270	0.424	0.3623
2	female	0.627	0.578	0.503	0.5693
3	female	0.784	0.575	0.832	0.7303
4	female	0.564	1.186	0.641	0.797
6	male	0.268	0.2235	0.290	0.2605
7	male	0.649	1.228	1.377	1.0847
8	male	0.907	0.714	0.945	0.8553
11	male	0.505	0.2435	0.218	0.3222
12	female	0.701	0.332	0.193	0.4087
13	male	0.112	0.364	0.246	0.2407
14	female	0.582	0.304	0.237	0.3743
15	male	0.112	0.109	0.1535	0.1248

Macronutrient Intake – Percentage of total kCal

	Total	*Male*	*Female*
CHO	48.52%	49.47%	46.67%
Protein	18.37%	18.31%	18.47%
Fat	33.98%	32.85%	36.18%

APPENDIX I

APELIN VAIRABILITY