THESIS

A ONE-WEEK CONTROLLED DIET AND EXERCISE INTERVENTION SIGNIFICANTLY IMPROVES INSULIN SENSITIVITY WITHOUT CHANGES IN PLASMA ADIPONECTIN CONCENTRATIONS IN YOUNG NON-HISPANIC WHITE AND MEXICAN AMERICAN ADULTS

Submitted by
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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MARIA FERNANDA BOTERO ENTITLED A ONE-WEEK CONTROLLED DIET AND EXERCISE INTERVENTION SIGNIFICANTLY IMPROVES INSULIN SENSITIVITY WITHOUT CHANGES IN PLASMA ADIPONECTIN CONCENTRATIONS IN YOUNG NON-HISPANIC WHITE AND MEXICAN AMERICAN ADULTS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

A ONE-WEEK CONTROLLED DIET AND EXERCISE INTERVENTION SIGNIFICANTLY IMPROVES INSULIN SENSITIVITY WITHOUT CHANGES IN PLASMA ADIPONECTIN CONCENTRATIONS IN YOUNG NON-HISPANIC WHITE AND MEXICAN AMERICAN ADULTS

Background: Hypoadiponectinemia (low levels of adiponectin in blood) has been linked to insulin resistance and type 2 diabetes (T2D). In the USA, the prevalence of these latter two conditions is higher in Mexican Americans (MA) when compared to Non-Hispanic Whites (NHW). Variations in plasma adiponectin concentrations may partially contribute to differences in insulin resistance and T2D prevalence rates between adults of these two ethnic groups. Also, plasma adiponectin concentrations in MA and NHW adults may respond differently to a diet and exercise intervention. Objectives: 1. To determine whether or not pre-intervention fasting plasma adiponectin concentrations differ between sedentary, non-obese MA and NHW adults; 2. To determine if a combined one-week exercise and diet intervention (controlled low-saturated fat, low-sugar, high-fiber diet) improves both plasma adiponectin concentrations and insulin sensitivity in both NHW and MA adults; 3. To determine if changes in insulin sensitivity are associated with changes in circulating adiponectin concentrations. Methods: During
the pre-study phase, volunteers underwent all initial screening tests. Eligible participants [n= 37; (20 NHW; 9 males, 11 females and 17 MA; 4 males, 13 females aged 18-40 years), fasting blood glucose < 126 mg/dl, blood pressure < 140/90 mm Hg, BMI < 30 kg/m^2] continued with a 7-day baseline period, in which they were asked to maintain their regular food intake and their usual low level of exercise. A 3-hour intra-venous glucose tolerance test (IVGTT) for the measurement of insulin sensitivity was performed at the end of this period, as well as measurement of fasting plasma adiponectin. The intervention phase started immediately the day after and ended 7 days later. Subjects consumed a diet that was rich in vegetables, fruits, dietary fiber, and lean proteins and low in saturated fat and refined carbohydrates. Study investigators prepared all foods which were provided to participants. Subjects also exercised on a stationary cycle ergometer for 6 out of the 7 days of the intervention (40-45min/session at 65%V02 max). At the end of the intervention, 16-17 hours after the last exercise bout, plasma adiponectin was again measured and another IVGTT was performed. Dependent variables were analyzed using a 2 X 2 repeated measures ANOVA. Results: Insulin action, determined by the 3-h insulin area under the curve (IAUC) in response to glucose infusion, improved significantly (p<0.05) in response to the intervention (IAUC µU*min/L pre, post: MA = 2297, 1635; NHW = 1794, 1210). At baseline, plasma adiponectin levels were not significantly different between NHW and MA adults (NHW= 11.42 mµ/ml, MA= 11.17 mµ/ml) and there were no significant changes in adiponectin in response to the intervention in either group. Conclusion: A one-week diet and exercise intervention significantly improved insulin action in both MA and NHW adults, but changes in circulating adiponectin were not observed. Thus, the improvement in insulin
sensitivity in response to a short-term diet/exercise intervention was disassociated from
any changes in circulating adiponectin.

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I have achieved one of my dreams in life. I realized what I want to be when I grow up: A Dietitian. It has been a long journey to become one and I need to thank the divine intelligence and all the people that in one way or another supported me, helped me, encouraged me and guided me throughout these years to make this dream a reality. A big thank you to Dr. Melby and Dr. Hickey for giving me the opportunity to be part of this research project and for all the guidance and knowledge acquired in the process. Thank you Dr. Melby for all your time and patience. Thank you Dr. Allen for being part of my thesis committee. Many other people participated in this research study and their help and support was invaluable, thank you Stacy, Katie and Holly.

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And like my father says: “FELICITARSEN!!!!!!!”
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Introduction and Hypothesis

Abdominal adiposity has been associated with increased risk of insulin resistance (IR) and type 2 diabetes mellitus (T2DM). In the United States, more than half of its population is estimated to have abdominal obesity. Mexican Americans (MA) make up the largest (64%) portion of the Hispanic/Latino population in this country and have a higher prevalence of abdominal adiposity when compared to their non-Hispanic White (NHW) counterparts. In the US, T2DM prevalence among Hispanics almost doubles (9.8%) the prevalence among NHW (5.0%) and it is estimated that almost half of the Hispanics born in the US in the year 2000 will be diagnosed with T2DM during their lifetime.

Increased abdominal adiposity is associated with higher systemic concentrations of pro-inflammatory adipocytokines like TNF alpha and IL-6 and lower circulating concentrations of an insulin sensitizing protein known as adiponectin. It has been found that adiponectin promotes lipid oxidation and glucose uptake in skeletal muscle and reduces glucose output in the liver. Hypoadiponectinemia (low levels of adiponectin in blood) has been linked to insulin resistance and T2DM and higher adiponectin levels, even in the population known to have the highest risk for T2DM of any population, the Pima Indians of Arizona, have shown protective effects.
Because of the positive effects of adiponectin on insulin sensitivity, the search for factors that could have an effect on endogenous adiponectin secretion and adiponectin action, especially in populations at risk, need to be further explored.

Some studies have examined adiponectin concentration variation on the basis of ethnicity. These studies have observed adiponectin level differences between the populations compared. This suggests that adiponectin concentrations may vary according to ethnic background. To my knowledge no studies have assessed adiponectin differences between normoglycemic, healthy, sedentary, non-obese young MA and NHW adults. Likewise the effects of a combined acute exercise training program and a healthy diet on plasma adiponectin levels in subjects with these characteristics are not known. In order to address these questions, the objectives of this study were the following:

1. To determine whether fasting plasma adiponectin concentrations at baseline differ between MA and NHW adults.

2. To determine if a combined one week exercise training program and healthy diet intervention will have an effect on plasma adiponectin levels and insulin sensitivity in both MA and NHW adults.

3. To determine if changes in insulin sensitivity are associated with changes in circulating adiponectin concentrations.
Chapter II

Literature Review

Obesity and its Prevalence Among the World and U.S. Population

Obesity has become the most common nutritional disorder in industrialized countries. Overweight and obesity cases have dramatically increased around the world and in the United States in the past two decades.\textsuperscript{16-18} The World Health Organization\textsuperscript{19} estimated that 1.6 billion adults (ages 15 and older) were overweight and at least 400 million adults were obese worldwide in 2005. These numbers will continue rising and by 2015 it is expected that 2.3 billion and more than 700 million adults will be overweight and obese, respectively.

In the United States, 33.3\% of men and 35.3\% of women were categorized as obese according to the National Health and Nutrition Examination Survey (NHANES) 2005-2006.\textsuperscript{20} The prevalence of obese adults in the United States remains high and there is no indication that it is decreasing. However, possibly the prevalence has reached a plateau, as there was no significant change in obesity prevalence for men or women between 2003-2004 and 2005-2006, based on NHANES data.\textsuperscript{20}

Obesity prevalence among men did not statistically change by race/ethnic group from 1999 to 2006.\textsuperscript{18, 20} In contrast, a significant race/ethnicity disparity can be observed among women. According to NHANES 2005-2006 data, more Mexican-American (MA)
women 40-59 years of age were obese (51%) when compared to their non-Hispanic white (NHW) (39%) counterparts of the same age. Similarly, data adjusted for NHANES period (1999-2000, 2001-2002, 2003-2004) indicates that MA women are more likely to have a BMI ≥ 30 when compared to NHW women (O.R. 1.31 (95% confidence interval: 1.11-1.55)).

**Energy Imbalance and its Economic Impact**

Obesity is a leading health concern in the United States. Obese adults are at a higher mortality risk when compared to normal weight subjects. Obesity is a multi-factorial disease that is characterized by energy imbalance. Overnutrition, physical inactivity, advances in technology, food consumption trends (dining out vs. at home), and decreases in tobacco consumption among many other social, cultural and economic factors may contribute to positive energy balance. The etiology of obesity is very complex and more definitive data on the association between changes in these behavioral and environmental factors and obesity prevalence rates are needed.

Obesity in the United States is not only considered a public health concern but is also associated with higher health care costs for those that suffer from this disease. Obese people in this country pay 36% more in inpatient and outpatient health care services when compared to normal weight people.

**Metabolic Syndrome**

The metabolic syndrome is a compendium of obesity-related abnormalities known as independent risk factors for cardiovascular disease (CVD) and type 2 diabetes mellitus
(T2DM). The Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, ATP III) established the following criteria as the components of the metabolic syndrome: central obesity (waist circumference > 102 cm for men and > 88 cm for women), hypertriglyceridemia (serum triglyceride levels ≥ 150 mg/dL), low high density lipoprotein cholesterol (HDL-C) with serum levels < 40 mg/dL in men and < 50 mg/dL in women, insulin resistance defined as serum glucose levels ≥ 110 mg/dL and blood pressure ≥ 130/85 mm Hg. An individual with three or more of these metabolic abnormalities is considered to exhibit the metabolic syndrome.

Ford et al. used the criteria described above to determine the prevalence of this syndrome among 8814 US adults of at least 20 years of age that participated in the NHANES III, 1988-1994. Age-adjusted results showed that 24% of men and 23.4% of women had the metabolic syndrome. Also, the risk of being diagnosed with the metabolic syndrome increases with advancing age in both men and women.

Mexican Americans (MA), who are the largest (population of 28.3 million as of 2006) and fastest growing Hispanic/Latino group in the United States had the highest prevalence of this syndrome (31.9%). Of this racial/ethnic group, women had a higher prevalence of the metabolic syndrome when compared to men, 35.6% and 28.3% respectively. MA women had consistently higher prevalence rates for every one of the five components of the metabolic syndrome included in the ATP III report when compared to NHW women. MA men had higher percentages of high blood pressure, hypertriglyceridemia and high fasting glucose than NHW men. Higher fasting glucose concentrations in both MA men and women translates into higher risk for insulin
resistance and T2DM development. In fact, according to a CDC report (selected areas, 1998-2002), the age-adjusted diabetes prevalence among Hispanics (9.8%) almost doubles the prevalence among NHW (5.0%).

Narayan et al. also confirmed the higher risk of developing T2DM among Hispanics. Their results indicate that Hispanics born in the year 2000 are the race/ethnic group in the United States that have the highest probability of being diagnosed with diabetes during their lifetime. These probabilities reach 45.4% if the individual is male and 52.65% if female. Understanding the reasons behind the higher rates for T2DM in MAs is critical to developing initiatives to lower the burden of this debilitating disease. A discussion of physiologic/metabolic factors linked to T2DM follows.

**Adipose Tissue**

The major known function of adipose tissue is to facilitate energy storage. There are two types of adipose tissue: white and brown. White adipose tissue (WAT) serves primarily in energy storage and is less metabolically active than brown adipose tissue (BAT), which uses stored triglycerides (TG) for heat production (thermogenesis).

Many different types of cells make up the adipose tissue. Some of these cells are: fibroblastic connective tissue cells, macrophages, preadipocytes and adipocytes. Adipocytes constitute the vast majority of the adipose tissue. These cells have the capacity to store energy in the form of triglycerides. Adipocytes’ content and therefore their size are constantly changing due to their ability to accumulate and release energy in both, short and long term. Adipocytes serve as a buffer for an energy imbalance when energy intake is not equal to energy output.
Another vital function of the adipose tissue is to act as an endocrine organ. In the past few years, several adipose-tissue derived proteins that have endocrine, paracrine and autocrine functions have been identified, including but not limited to, leptin, interleukin 6 (IL-6), tumor necrosis factor (TNF) alpha, and adiponectin.

Adipocytes, via their secreted proteins, modulate energy homeostasis and glucose and lipid metabolism by sending efferent signals to and receiving afferent signals from central (brain) and peripheral (muscle and liver) targets. In healthy humans, leptin, one of these secreted proteins, communicates with the central nervous system (CNS) to inhibit food intake and increase energy expenditure when its levels rise during the overfed state. As leptin levels decline during fasting, energy expenditure is reduced and feeding is stimulated. This complex and sophisticated feedback system plays a major role in the regulation of energy homeostasis. Adipocyte secreted proteins share common signaling pathways to communicate with the central and peripheral tissues but also have distinct ways to interact with these targets. Disturbances in receiving or sending these messages lead to disruption of proper body function, energy homeostasis and adverse metabolic consequences.

**Adipose Tissue Distribution**

Body mass index (BMI), waist to hip ratio (WHR) and waist circumference (WC) are anthropometric measurements used to estimate total and visceral adiposity. BMI, calculated as the weight in kilograms divided by the square of the height in meters (kg/m²) is an index widely used in the United States and around the world to estimate general adiposity in adult individuals. Both the overweight and obese categories are
physiological states characterized by an abnormal accumulation of body fat. Overweight is defined as a BMI of 25 to 29.9 kg/m$^2$ while obese is defined as a BMI of 30 kg/m$^2$ or greater. On the other hand, visceral adiposity is defined as the accumulation of fat around the viscera and inside the intra-abdominal solid organs. Although overall obesity diagnosed by BMI and visceral obesity diagnosed by WC and WHR predict the risk of T2DM, visceral adipose tissue is more strongly linked to insulin resistance, T2DM, and CVD than peripheral (gluteal/subcutaneous) adiposity. Metabolic activity in the visceral region appears to be higher when compared to the activity of the subcutaneous gluteo-femoral area.

Visceral obesity is also considered an independent cardiovascular risk factor even in individuals in which overall obesity (BMI) lies within normal ranges. Excess visceral fat accumulation leads to hepatic and adipose tissue insulin resistance, glucose intolerance, low HDL-cholesterol, hypertension and hypertriglyceridemia. Also, the development of insulin resistance and coronary calcification among non-diabetic asymptomatic men and women was strongly associated with visceral fat accumulation.

In addition to WHR and WC, magnetic resonance imaging (MRI) and computerized tomography (CT) are also effective tools to assess visceral fat content. In fact, the evidence report expert panel highlights the MRI and CT as the gold standard for visceral adiposity assessment. The downsides of using these two diagnostic tools are that cost is high and availability is limited. This is why the use of WC as the most appropriate tool for visceral fat assessment in a clinical setting is recommended. Waist circumference has been strongly associated with all obesity-related complications and is also one of the components of the clinical criteria for metabolic syndrome diagnosis.
Among U.S. adults the prevalence of visceral adiposity is greater than the prevalence of obese individuals (BMI ≥ 30 kg/m²).\textsuperscript{17-18} As defined by the ATP III, more than one half of U.S. adults had visceral obesity during 2003-2004.\textsuperscript{1} Increasing trends and current prevalence of visceral obesity and its strong association with T2DM and cardiovascular disease may be a key predictor of future morbidity and mortality among adults in the United States.\textsuperscript{1}

**Visceral Adiposity and Insulin Resistance**

The link between visceral adiposity and insulin resistance has not been fully elucidated. Nevertheless there are two popular hypotheses among researchers that may explain this association. The first and oldest one is known as the lipotoxicity theory.\textsuperscript{43} This theory states that intra-abdominal adipocytes are more lipolytically active\textsuperscript{39}. This increased activity and adipocyte hypertrophy\textsuperscript{44} results in an elevated mobilization of free fatty acids (FFA) into portal circulation and to the liver.\textsuperscript{39,45} Activation of beta-3 adrenergic receptors expressed in visceral adipocytes may be responsible for the increased lipolysis rate.\textsuperscript{45-46} Elevated liver FFA influx overloads hepatocytes with lipid content resulting in increased de novo TG synthesis. Triglyceride accumulation in the liver (hepatic steatosis), leads to a condition known as non-alcoholic fatty liver disease (NAFLD).\textsuperscript{47-48} NAFLD diagnosis requires that hepatic lipid content be 5% to 10% by weight in the absence of excess alcohol consumption (> 20 g/d).\textsuperscript{47} Results from a multiethnic population-based study (race/ethnicity distribution: 32.1% white, 48.3% black, and 17.5% Hispanic) showed that almost one third of the population suffered from hepatic steatosis and that the prevalence of this condition in Hispanics is greater (45%)
when compared to both, whites (33%) and blacks (24%). In addition, increased hepatic FFA disposal into circulation in the form of TGs via VLDL particles exacerbates dyslipidemia. Excess FFA flux to the liver also results in hyperinsulinemia. Approximately 50% of the pancreas-secreted insulin is cleared by the liver on the first passage before it reaches peripheral circulation. FFA accumulation in the liver reduces its insulin extraction capacity contributing to increased insulin levels in circulation. Also, increased portal FFA flux has been associated with increased gluconeogenic (GNG) flux and increased liver fat has been associated with hepatic insulin resistance.

The second and most recent hypothesis is that visceral adipocytes and their associated macrophages secrete a series of proteins termed adipocytokines. Increased visceral adiposity may then lead to insulin resistance through alterations in the secretion of these adipocyte-derived proteins. Secretion of pro-inflammatory adipocytokines like TNF alpha and IL-6 is up-regulated (i.e. higher rates of secretion associated with higher levels of visceral adiposity) while the secretion of adiponectin, an insulin sensitizing protein is down regulated (i.e. higher rates of secretion occur with lower levels of adiposity).

**Adiponectin: a Novel Adipocytokine**

Adiponectin is a recently discovered (1995) 30-kDa adipocytokine also referred as gelatin-binding protein-28 (GBP-28) and adipose most abundant gene transcript-1 (apM1). The mouse homolog of adiponectin has been cloned as adipocyte complement-related protein of 30-kDa (Acrp30) and is also referred to as AdipoQ.
Adiponectin is a protein that is apparently exclusively produced by adipocytes and constitutes the most abundant protein in plasma. This protein, accounts for 0.01% of total plasma protein. Adiponectin plasma range in human subjects is 3-30 micrograms/ml with an average of 5 to 10 micrograms/ml.

In plasma, adiponectin can be found as multimeric complexes (trimers, hexamers and a high-molecular-weight (HMW) form) or as a globular domain. The latter is found in very small amounts.

**Figure 2.1. Domain and Structure of Adiponectin**

A full length single adiponectin molecule is composed of 244 amino acids. It includes a collagen-like fibrous domain at the N-terminus, a variable region domain and a C1q-like globular domain at the C-terminus. Full length adiponectin combines in plasma via its collagen domain to create the multimeric complexes mentioned above. Hexamers of ~190 kDa and HMW multimers > 300 kDa circulate in larger amounts. HMW adiponectin appears to be the most metabolically significant multimeric form.
Nevertheless the extent of how adiponectin multimeric forms may exert different biological effects remains unclear.\textsuperscript{64}

**Adiponectin Receptors**

Adiponectin (full length and globular forms) binds to two types of receptors: AdipoR1 and AdipoR2.\textsuperscript{65} AdipoR1 is highly expressed in mouse skeletal muscle and has a higher affinity for globular adiponectin. AdipoR2 is most abundantly expressed in mouse liver and its affinity for the full-length adiponectin is higher.\textsuperscript{65} Contrary to the mouse pattern, Civitarese et al.,\textsuperscript{66} reported mRNA expression of both AdipoR1 and AdipoR2 in skeletal muscle of non-diabetic Mexican Americans with or without a family history of type 2 diabetes. Insulin sensitivity in these subjects was positively correlated with the expression levels of these receptors.

Both, AdipoR1 and AdipoR2 have the ability to activate signaling molecules such as AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor (PPAR) alpha, and p38 mitogen-activated protein kinase (MAPK) in vitro.\textsuperscript{65} Activation of AMPK by adiponectin is impaired in skeletal muscle of ob/ob mice. These insulin resistant mice exhibit reduced AdipoR1 and AdipoR2 expression levels. The hyperinsulinemic state of these ob/ob mice reduces the expression of these two receptors.\textsuperscript{67} Decreased expression levels of AdipoR1 and AdipoR2 reduces adiponectin sensitivity, which in turn worsens insulin resistance.\textsuperscript{67}

In addition to AdipoR1 and R2, T-cadherin (a glycosylphosphatidylinositol-anchored extracellular protein) may act as a receptor for hexameric and HMW forms of adiponectin.\textsuperscript{68}
Adiponectin Mechanisms of Action

Insulin-Sensitizing properties

Increased activation of both AMPK and PPAR alpha in muscle and liver may contribute to insulin sensitivity. In the liver, AMPK decreases glucose output through decreased gluconeogenesis. Isolated hepatocytes treated with Acrp30 (full-length adiponectin) increased the ability of sub-physiological levels of insulin to suppress glucose output. Similarly, a drop in serum glucose was observed when mice where treated with Acrp30 injections. This experiment was performed near basal conditions to make sure insulin levels where not affecting glucose uptake. Serum glucose was primarily determined by hepatic glucose output. In the liver, AMPK also increases fatty acid Beta-oxidation.
through the phosphorylation and subsequent deactivation of Acetyl Coenzyme A Carboxylase (ACC). Increased expression of PPAR alpha also promotes hepatic fatty acid Beta-oxidation.

Similarly, in muscle, fatty acid Beta-oxidation results as a consequence of AMPK and PPAR-alpha activation. PPAR alpha increases the expression level of molecules such as CD36 involved in fatty-acid transport into tissues. Additionally, AMPK increases GLUT4 translocation which translates in increased glucose uptake.

Increased FFA oxidation in muscle reduces TG content. Decreased muscle TG content might contribute to the improvement of insulin signal transduction. In agreement with this statement, Fruebis, et al. reported a significant decrease in plasma FFA, glucose and TG concentration in lean mice treated with gAcrp30 after being fed with a high fat test meal. No significant changes in insulin or glucagon were observed. This group of researchers reported that the decline in plasma FFA concentration or a possible reduction in FFA metabolites as a consequence of a higher rate of FFA oxidation in muscle may explain the reduction in plasma glucose levels. In contrast, a recent study reported that fatty acid-induced insulin resistance in humans improves instead of declining, in the presence of intra muscular TG synthesis and storage by reducing the availability of fatty acid metabolites.

Activation of AMPK by the different biological adiponectin forms is still controversial, Yamauchi et al. reported that both full length and globular adiponectin forms were able to activate AMPK in muscle and only full-length adiponectin was capable to do so in the liver. Contrary to these findings, Tomas, et al. concluded that only globular adiponectin activates AMPK in skeletal muscle.
The direct effect of adiponectin on tyrosine phosphorylation of the insulin receptor (IR) has been postulated as another mechanism for its insulin-sensitizing properties. In a cross-sectional study, low fasting plasma adiponectin concentration in humans was associated with a high basal skeletal muscle tyrosine phosphorylation of the IR. In this study, researchers state that hyperinsulinemia associated with low plasma adiponectin concentrations may increase basal phosphorylation of the IR. High basal IR tyrosine phosphorylation decreases skeletal muscle IR phosphorylation capacity upon insulin stimulation reducing its ability to initiate the insulin-signaling cascade and therefore decreasing insulin sensitivity. Prospectively, low adiponectin plasma concentration at baseline was associated with a decrease in insulin sensitivity.
Anti-atherosclerotic properties

Adiponectin has the ability to prevent the development of atherosclerotic lesions by inhibiting the following cellular phenomena:

1. Monocyte adhesion to endothelial cells

   Pro-inflammatory cytokines like TNF-alpha activate the vascular endothelium to facilitate monocyte adhesion. Pro-inflammatory cytokines like TNF-alpha activate the vascular endothelium to facilitate monocyte adhesion. Physiological concentrations of adiponectin were shown to strongly inhibit TNF-alpha induced expression of intracellular adhesion molecules such as vascular cellular adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM), and E-selectin.

2. Expression of class A-1 scavenger receptor of macrophages

   Adiponectin decreases uptake of oxidized low-density lipoprotein (LDL) by macrophages and also decreases subsequent foam cell formation by inhibiting the expression of macrophages’ class A-1 scavenger receptors.

3. Proliferation and migration of smooth muscle cells

   In cultured smooth muscle cells, Adiponectin suppresses the proliferation and migration of smooth muscle cells via direct binding to platelet-derived growth factor-BB (PDGF-BB) and the inhibition of signal transduction through extracellular signal related kinase (ERK). The inhibitory cellular mechanisms mentioned above, reinforce the idea that Adiponectin may have anti-atherosclerotic functions.
Anti-inflammatory properties

Adiponectin suppresses the production of TNF- alpha and C-reactive protein (CRP) in adipose tissue. Increased expression of both TNF- alpha and CRP are present during systemic inflammation. In human adipose tissue, CRP mRNA expression was inversely correlated with adiponectin mRNA expression (r = -0.29, P < 0.01). Inhibition of lipopolysaccharide induced-TNF-alpha production and phagocytic activity was observed in cultured macrophages treated with adiponectin.

Figure 2.4. Role of Adiponectin as an Anti-Atherogenic and Anti-Inflammatory Molecule.

Okamoto, Y. et al. Clinical Science 2006;110:267-278
Effect of Lifestyle Modifications on Adiponectin Levels

Several studies have shown an inverse significant correlation between insulin resistance, T2DM and adiponectin levels. To date, the most appropriate strategy to positively alter adiponectin plasma levels is still debatable. Exercise and diet only approaches have been studied, as well as a combination of both factors in short and long-term study designs.

Exercise

Several studies exploring the effects of exercise on plasma adiponectin levels have resulted in inconsistent findings. Kriketos et al., reported a 260% increase of adiponectin levels after two to three bouts of exercise (~ 1 week) (7.0 +/- 0.7 vs. 18.2 +/- 1.9 µg/ml, p< 0.0001), despite unchanged body weight in overweight sedentary Australian males. After 10 weeks of aerobic exercise 4-5 days per week for 40 minutes per session, no significant body fat mass change was recorded and adiponectin levels remained elevated (16.4 +/- 1.9 µg/ml). Kondo et al., also reported an adiponectin level increase of 42.8% after a 7 month moderate intensity exercise training program in healthy, obese young Japanese women. In this study, exercise resulted in significant body fat mass reduction (p<0.05).

In contrast, Hulver et al., concluded that adiponectin levels are not altered with exercise training despite enhanced insulin action (SI + 98%) after a 6 month-endurance exercise program in sedentary healthy male and female subjects. Body mass remain unchanged after the exercise intervention. Rockling-Andersen et al., also reported no significant effect of exercise on circulating adiponectin levels in Norwegian men with
several risk factors for diabetes and cardiovascular disease after a 1 year exercise training program.

Diet

A recent prospective and cross-sectional evaluation of plasma adiponectin concentrations and dietary data from 987 diabetic women with no history of cardiovascular disease from the Nurses’ Health Study concluded that high adherence to a Mediterranean-type diet resulted in increased median plasma adiponectin concentrations by 23%. These women consumed more alcohol, fish, fruit, legumes, nuts, vegetables, whole grains and had a higher ratio of polyunsaturated to saturated fat, ate less red and processed meats, and had a higher total cereal, fruit, and vegetable fiber intakes when compared to low adherers. Of all of the above components of the Mediterranean diet, alcohol, nuts and whole grains had the strongest association with adiponectin plasma levels. 89

Another study on nondiabetic and diabetic obese Japanese men and women reported increased adiponectin concentrations accompanied with significant weight loss after a 2 month calorie-restricted diet. Plasma adiponectin concentration significantly increased in the nondiabetic (42 +/- 13%, p <0.01) and diabetic subjects (65 +/- 22%, p <0.05) after a 10 +/- 1% and 12 +/-2% BMI reduction in the nondiabetic and diabetic subjects respectively. 9

Exercise and Diet

Other studies have reported increased adiponectin levels after diet-induced and physical activity weight loss interventions. Ounis et al. 90 reported an increase in adiponectin levels of 51.6% above baseline after a 2 month hypocaloric diet and exercise
intervention in Tunisian female obese adolescents. This increase in adiponectin was accompanied with a substantial body fat mass loss (19.6% of initial body fat mass). Changes in adiponectin levels in the diet and exercise training only groups were lower, 23% and 38.5% respectively, when compared to the additive effects of diet and exercise. Body fat mass change in the exercise group was not statistically significant.

Similarly, a significant (p<0.001) body fat mass reduction of ~10% of baseline BMI achieved by obese premenopausal Italian women after adherence to a low-energy Mediterranean-style diet and increased physical activity program for two years was followed by a significant (p< 0.01) increase in plasma adiponectin concentrations (2.2 µg/ml).85

The effects of lifestyle modifications on adiponectin levels are not definitive. The effect of exercise on adiponectin is especially contradictory. More research focusing on exercise, diet, or the combination of both as potential modifiable factors of plasma adiponectin concentrations is needed.

**Hypoadiponectinemia**

Unlike other adipose-derived hormones, adiponectin mRNA is down-regulated in visceral adipose tissue of both lean and obese subjects.91 Hypoadiponectinemia has been associated with increased risk of cardiovascular disease, obesity as well as metabolic diseases like T2DM.9, 92

Matsuzawa61 has proposed a disease entity named hypoadiponectinemia. Two types of hypoadiponectinemia have been proposed: primary and secondary. The first one is associated with genetic disorders.61 Japanese subjects with T2DM and age and BMI-
matched nondiabetic control subjects were screened for mutations in the adiponectin gene. In adiponectin’s globular domain, researchers identified four missense mutations: R112C, I164T, R221S and H241P. Patients with T2DM had a significantly higher frequency of the I164T mutation when compared with their controls (p < 0.01). Subjects carrying I164T mutation had lower plasma adiponectin concentrations than subjects not expressing the mutation. Traits of the metabolic syndrome, including hypertension, hyperlipidemia, T2DM and atherosclerosis were exhibited by all the subjects with I164T mutation.\textsuperscript{93} Also, single nucleotide polymorphism SNP276 of the adiponectin gene has been found to be associated with insulin resistance, IGT and hypoadiponectinemia.\textsuperscript{94-95}

The genetic basis of plasma adiponectin in Hispanic-American and African-American families, all members of the Insulin Resistance Atherosclerosis Family Study (IRAS study) was also assessed. Researchers identified a major quantitative trait loci in Hispanic-Americans on chromosome 3q27 (region where the adiponectin gene is located) that contributes to the variation of plasma adiponectin levels in this population. This phenomenon was not observed in the African-American families.\textsuperscript{96}

Other studies have mapped one of the quantitative trait loci affecting insulin resistance/metabolic syndrome\textsuperscript{97} and T2DM\textsuperscript{98} on this same chromosome (3q27). These findings may indicate a possible association between the etiology of these disorders and adiponectin.\textsuperscript{93} Also, fasting serum adiponectin levels in Hispanic children from the Viva La Familia study were found to have a high heritability factor. In these children, genes accounted for 93\% of the variance of adiponectin circulating levels.\textsuperscript{99}
Secondary hypoadiponectinemia may have its root cause in the accumulation of visceral adiposity.\textsuperscript{61} The prevalence of secondary hypoadiponectinemia is higher than the primary one.\textsuperscript{61}

**Hypoadiponectinemia, insulin resistance and T2DM**

Hypoadiponectinemia predicts the future development of insulin resistance,\textsuperscript{9,74,100} T2DM,\textsuperscript{11,101-102} and metabolic syndrome\textsuperscript{103-104} in diverse ethnic groups and geographical areas.

In humans, low circulating adiponectin levels associated with hyperinsulinemia have been linked with decreased insulin sensitivity via reduced insulin receptor tyrosine phosphorylation in skeletal muscle upon insulin stimulation.\textsuperscript{74} A recent study suggests that hypoadiponectinemia may be one of the predictors of abnormal glucose homeostasis in patients having impaired glucose tolerance (IGT).\textsuperscript{105} Initial comparisons of plasma adiponectin concentrations between Japanese diabetic men and women subjects and age and BMI-matched control group showed that subjects with diabetes had lower plasma adiponectin concentrations (p < 0.001).\textsuperscript{9} In the same population a 5 year follow-up study concluded that subjects with serum levels of adiponectin in the lowest tertile developed diabetes 9.3 times more often than those in the highest tertile.\textsuperscript{101} Similarly, another Japanese study of diabetic men concluded that insulin resistance associated with visceral fat accumulation may be mediated by lower adiponectin concentrations.\textsuperscript{106}

Adiponectin levels have been also studied in the population with the highest known prevalence of T2DM of any population, the Pima Indians of Arizona, USA.\textsuperscript{11} This population is also prone to obesity. Seventy cases (patients who later developed T2DM)
and seventy controls matched for BMI, age and sex were taken from the longitudinal study of health in the Pima Indian Population. Results showed that at baseline, subjects in the cases group had lower adiponectin concentrations than subjects in the control group (p=0.01). Subjects with higher adiponectin concentrations had a protective effect against diabetes making them less likely to develop T2DM when compared to subjects with low concentrations of this protein (incidence rate ratio 0.63 (95% CI 0.43-0.92); p =0.02).\textsuperscript{11} In Mexican children high adiponectin concentrations also predicted a lower prevalence of T2DM (odds ratio 0.86, p=0.001) independent of sex, age, and BMI (R2=0.318, p<0.001).\textsuperscript{107}
Chapter III
Methods and Procedures

Subjects

The date set for this study is the same one used for a dissertation (Dr. Stacy Schmidt, Colorado State University, 2009), which examined cardiometabolic plasticity in MA and NHW. The insulin action and blood lipid data were part of this dissertation. The present thesis extends the study findings to include the adiponectin values and their relation to insulin action and the blood lipids. This study included a total of 37 sedentary, non-obese, apparently healthy, male and female participants. Of these 37 individuals, 20 were non-Hispanic whites (NHW) and 17 were Mexican Americans (MA). Ethnic background was determined based on at least 3 grandparents being either from Mexico (i.e. the Mexican Americans) or of European Caucasian descent (i.e. the non-Hispanic whites). This information was obtained from the ethnicity questionnaire (Appendix B).

The eligibility of each study participant was determined after performing a series of initial screening tests and questionnaires. Exclusionary criteria included the following: diabetes (fasting blood glucose level > 126 mg/dl), hypertension (blood pressure > 140/90 mm Hg), use of tobacco products, pregnant or following a vegetarian diet, history of eating or endocrine disorders, use of medications that could potentially have an effect on the variables measured in this study (insulin action and certain pro-inflammatory and anti-inflammatory cytokines), weight unstable (>2.5 kg change over the previous six months), body mass index (BMI) > 30 kg/m² and engaging in structured, intentional
exercise more than once a week during the immediate past year. Additionally, eligibility was limited to subjects aged 18–40 years. Eligible participants were asked to commit to the study for about 20 hours during a 19-day period. During the baseline period, which was the first 7 days of the study, subjects were asked to maintain their regular food intake and their usual low level of exercise. During the intervention period, the second week of the study, participants completed six monitored exercise sessions (one session per day, with one day of rest) and consume the food prepared and provided to them by the research team. Before and after the intervention period, subjects underwent an intravenous glucose tolerance test (IVGTT) for measurement of insulin sensitivity.

The protocol for this study was approved by the Colorado State University Human Research Committee prior to the beginning of the study. All participants were informed of the protocol, procedures, benefits and possible risks associated with their participation in this study. Verbal and written consent was obtained from each participating subject (Appendix A).

**Anthropometric Measurements and Body Composition**

Body weight was determined to the nearest 100g on a physicians balance scale. Body height was measured to the nearest 0.1 cm with a wall–mounted stadiometer. A non-stretchable tape measure was used to determine waist and hip circumferences, with the tape position horizontally at the greater hip trochanter for the hip circumference and at the narrowest portion of the waist for the waist circumference for women and at the level of the umbilicus for men. These dimensions were measured to the nearest 0.1 cm. A
dual-energy x-ray absorptiometry (DEXA) (Model DPX-IQ Lunar Corp., Madison, WI) was used to assess absolute fat mass, fat-free mass and percentage of body fat.

**Resting Metabolic Rate and Total Daily Energy Expenditure**

To measure resting metabolic rate (RMR), subjects were asked to report to the research laboratory early in the morning after a 12-hour overnight fast. RMR was measured for a 30-minute period by indirect calorimetry. Subjects were asked to lie comfortably on a bed, avoid fidgeting, and refrain from falling asleep while wearing a nose clip and breathing through a mouthpiece. This mouthpiece was connected to a metabolic cart (CPX Express, Med Graphics, St. Paul, MN) which was used to measure oxygen consumption (VO\(_2\)) and carbon dioxide production (VCO\(_2\)). Respiratory gas exchange values were used to determine caloric expenditure using the Weir equation. To maintain subjects weight stable throughout the study, total daily caloric needs were determined based on the total daily energy expenditure calculated for a sedentary person plus the energy cost of the daily exercise bout.

**Cardiorespiratory Fitness Test**

Subject’s cardiovascular fitness level was determined by measuring the volume of oxygen consumed while exercising at maximum capacity (maximal oxygen consumption or VO\(_2\) max). A graded exercise test on a stationary cycle ergometer was performed. Exercise workload was set to begin at 25 watts after a warm-up period and gradually was increased in 25 watts increments every minute until subject reached exhaustion. While exercising, an open circuit spirometer was used to measure total oxygen consumption,
carbon dioxide production, pulmonary ventilation, and respiratory exchange ratio (RER). Additionally, subject’s heart rate was measured and monitored by using a Polar heart rate monitor.

**Pre-Intervention Dietary Assessment**

During baseline period, subjects were trained to report food intake data in real time for 3 consecutive days. When submitted to the research team, food intake records were analyzed using FIAS (Food Intake Analysis Software version 3, University of Texas Health Sciences Center, School of Public Health, Houston, 1998). Total energy, macronutrients (fat, saturated fat, carbohydrate, protein) and fiber intake were assessed.

**Diet Composition and Exercise During Intervention**

Subjects were expected to eat all meals, beverages and snacks provided to them during the 7 days of the intervention period. Emphasis was made on eating no more or less of what was provided. Study participants were required to pick up foods three times during this week and food satisfaction was assessed at this time. The experimental diet was low in saturated fat (low-fat dairy, lean meats) and refined sugars and was rich in whole grains, fruits and vegetables. Meal plans during the intervention period were designed to have the following diet composition: 50, 30, and 20 percent of energy as carbohydrate, fat, and protein respectively. Saturated fat was set to be lower than 5% of total kcalories. FIAS was used to create meal plans.

Exercise sessions were scheduled according to each subject’s time availability for 6 out of the 7 days of the intervention phase. Subjects exercised for 40 minutes for the first three days of exercise and 45 minutes for the last three days of exercise at a heart
rate that matched an exercise intensity of 65% of the individual’s VO₂ max. Trained personnel closely monitored heart rate throughout each session. The last exercise session was always undertaken the day prior to the second IVGTT used to determine insulin sensitivity.

**Intravenous Glucose Tolerance Test (IVGTT)**

An IVGTT to assess insulin sensitivity (how well the body responds to insulin) was performed twice during the course of the study, once at the end of the 7-day baseline period in which the subjects maintained their usual diets and sedentary lifestyle, and once again after the 7 day diet-exercise intervention. Subjects were asked to report to the Hartshorn Health Center after a 12-hour overnight fast before and after the intervention phase. This health center is located on the premises of the Colorado State University Campus. Subjects were asked to lie on a bed and trained personnel placed a catheter in an antecubital vein. Two baseline samples (~ 5 ml each) were withdrawn before glucose load administration. Both, fasting glucose and fasting insulin were measured from these samples. These data were subsequently used to assess insulin resistance using the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR was determined: HOMA-IR = [insulin (μU/mL) x glucose (mmol/L)/22.5]. At time 0 minutes, a 50% dextrose solution (0.3g/kg of body weight) was infused over 90 seconds. Then, 14 blood samples (~ ½ ml per sample) were collected at 2, 4, 8, 19, 22, 30, 40, 50, 58, 63, 70, 100, 140, and 180 minutes. All samples were immediately placed on ice until centrifuged after the test was completed. Plasma was removed from the centrifuged blood samples, placed in bullet tubes, and stored at minus 70° C for later analysis. Plasma
samples were sent to the University of Colorado Health Sciences Center General Clinical Research Center for insulin concentration measurements. An enzyme-linked immunosorbent assay (ELISA) kit was used to determine insulin levels. Glucose concentrations were measured using the glucose oxidase method on an automated glucose analyzer (YSI 2300, YSI Inc. Yellow Springs, Ohio). The area under the 3-h response curve (AUC) was calculated for glucose and insulin based on the trapezoidal method.

**Adiponectin Assay**

Fasting plasma samples (pre and post intervention) were used to measure plasma adiponectin concentrations. Total plasma adiponectin concentrations were measured at the Nutrient Biochemistry laboratory in the Food Science and Human Nutrition Department at Colorado State University using the Millipore Human Adiponectin ELISA kit # EZHADP-61k.

**Statistical Analysis**

For the analysis of data, the SPSS software was used. The dependent variables were analyzed for interactions and main effects using a two-way analysis of variance (ANOVA) [ethnicity (MA vs NHW) x time (pre- versus post-intervention)]. Significant associations between variables were determined using Pearson Product Moment correlations. Statistical significance was determined at p value < 0.05.
Chapter IV

Results

The baseline physical characteristics of the 37 subjects that participated in this study are shown in Table 4.1. Despite similar BMI in both the NHW and MA groups, there were significant (p <0.05) weight and height differences between ethnicities. No other differences were found between ethnicities.

Table 4.1. Physical Characteristics of the Study Participants at Baseline

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-Hispanic Whites</th>
<th>Mexican Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=9 Males</td>
<td>n=11 Females</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.8±2.4</td>
<td>24.0±5.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.2±9.8</td>
<td>71.2±9.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181.2±8.3</td>
<td>168.9±6.4</td>
</tr>
<tr>
<td>BMI (m²/kg)</td>
<td>26.2±1.7</td>
<td>25.0±2.5</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>20.4±2.6</td>
<td>35.0±5.3</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>86.5±5.4</td>
<td>76.8±8.6</td>
</tr>
<tr>
<td>VO₂ Max (ml/kg/min)</td>
<td>38.9±5.3</td>
<td>29.4±4.1</td>
</tr>
</tbody>
</table>

*= Significant difference between NHW and MA, p <0.05
Values are mean ± SE

Diet characteristics at baseline and during the intervention phase are shown in Table 4.2. At baseline, no significant dietary differences were observed between
ethnicities. When baseline and intervention diets were compared, significant dietary changes were found for all dietary components measured, except for CHO (% kcal) intake for both NHW and MA. Total kilocalories were significantly higher during the intervention, which was expected given the additional calories required to keep the subjects weight stable in the face of the increased energy expenditure from exercise.

Table 4.2. Diet Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Self-reported)</td>
<td>(Research Diet)</td>
</tr>
<tr>
<td></td>
<td>NHW (n=20)</td>
<td>MA (n=17)</td>
</tr>
<tr>
<td>Total Kcal</td>
<td>2238±582</td>
<td>1889±662</td>
</tr>
<tr>
<td>CHO (% kcal)</td>
<td>49.3±9.3</td>
<td>46.7±7.5</td>
</tr>
<tr>
<td>Fat (% kcal)</td>
<td>32.0±6.0</td>
<td>35.2±3.4</td>
</tr>
<tr>
<td>Sat Fat (% kcal)</td>
<td>11.6±2.7</td>
<td>11.9±2.5</td>
</tr>
<tr>
<td>Prot (% kcal)</td>
<td>15.2±3.2</td>
<td>15.5±3.8</td>
</tr>
<tr>
<td>Fiber (grams)</td>
<td>16.6±5.6</td>
<td>13.2±5.8</td>
</tr>
</tbody>
</table>

* = Significantly different from NHW during intervention, p <0.05
° = Intervention different from baseline within ethnicity, p <0.05
Values are mean ± SE

Both, total fat (% kcal) and saturated fat (% kcal) intake significantly decreased during the intervention. Total fat was reduced from 32.0±6.0 % kcal for NHW and 35.2± 6.4% kcal for MA to ~26% Kcal for both ethnicities. Saturated fat (% kcal) decreased from ~12% to ~5% for both NHW and MA. Protein and fiber intake were significantly higher during the intervention phase. Protein intake increased from ~15% to ~22% in both groups. Fiber consumption went up from 16.6±5.6 grams per day for NHW and 13.2±5.8 grams per day for MA to 45.8±9.8 grams in NHW and 41.0±8.1 in MA. A significant
total kcal difference was observed between ethnicities during intervention. Total daily caloric needs were significantly higher in NHW than in MA.

Despite adding additional calories to subjects’ diets to compensate for the additional energy cost of exercise, a 0.5 kg significant weight reduction was observed from pre-to post intervention across both groups.

There was a significant (p<0.05) main effect for time for HOMA-IR pre-post intervention. Insulin resistance decreased in both groups after the exercise and diet treatment as shown in Figure 4.1. HOMA-IR was higher in MA when compared to NHW at both baseline and post -intervention periods.

**Figure 4.1 HOMA-IR Pre and Post Intervention**

There was a significant main effect for time for Insulin AUC with values decreasing pre-to-post, which is indicative of an improvement in insulin sensitivity. A
significant main effect of ethnicity was also observed, with MA exhibiting a greater IAUC compared to NHW. As shown in Figure 4.2, there was no ethnicity by time interaction, indicating similar proportional improvements in insulin sensitivity in both groups with the diet-exercise intervention.

**Figure 4.2 Insulin AUC Pre and Post Intervention**

![Graph showing Insulin AUC Pre and Post Intervention](image)

- ● = Significant time effect pre-post intervention, p < 0.05
- ★ = Main effect of ethnicity, MA > NHW, p<0.05
- NHW n=19, MA n=14
- Values are mean ± SE
- Covaried for sex

At baseline, MA and NHW had similar plasma adiponectin concentrations with MA adiponectin concentration being slightly lower than NHW. However there were no significant main effects of time or ethnicity observed as shown in Figure 4.3. When baseline plasma adiponectin concentration in the two ethnic groups of women were compared, the concentration in MA women was 13% lower when compared to their NHW counterparts, 11.54±3.7 vs 13.26±5.3. However, this difference was not statistically significant.
Figure 4. 3. Adiponectin Plasma Concentration Pre and Post Intervention

No main effect of time or ethnicity were observed
NHW n=19, MA n=17
Values are mean ± SE
Covaried for sex

Correlation and partial correlation analyses were run to establish the strength and direction of the relationships between plasma adiponectin at baseline and several other pre-test variables. Pre-adiponectin was significantly negatively correlated with Ins AUC and HOMA-IR and significantly positively correlated with HDL-cholesterol as shown in Table 4.3. A significant association between pre-adiponectin and WHR and waist circumference was not observed.

Table 4.3. Significant Correlations of Pre Intervention Adiponectin

<table>
<thead>
<tr>
<th></th>
<th>HOMA-IR(pre)</th>
<th>Insulin AUC(pre)</th>
<th>HDL-chol(pre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson (r)</td>
<td>-0.383</td>
<td>-0.415</td>
<td>0.542</td>
</tr>
<tr>
<td>P value</td>
<td>0.021</td>
<td>0.018</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Significant correlations p< 0.05
A partial correlation analysis while controlling for gender and ethnicity lowered the strength of the relationship between pre-adiponectin and Ins AUC and HOMA-IR making the association for neither of the pairs compared non-significant as shown in Table 4.4. This data suggests that both gender and ethnicity have an effect on the relationship between pre-adiponectin and the insulin sensitivity markers mentioned above. At baseline MA had lower adiponectin levels and higher HOMA-IR and insulin AUC. On the other hand, NHW had higher adiponectin levels and lower HOMA-IR and Insulin AUC. The only association that remained significant after controlling for gender and ethnicity was the relationship between pre-adiponectin and pre-HDL-cholesterol (Table 4.4).

**Table 4.4. Partial correlations of pre intervention adiponectin while controlling for gender and ethnicity**

<table>
<thead>
<tr>
<th></th>
<th>HOMA-IR(pre)</th>
<th>Insulin AUC (pre)</th>
<th>HDL-chol(pre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial r</td>
<td>-0.308</td>
<td>-0.317</td>
<td>0.492</td>
</tr>
<tr>
<td>P value</td>
<td>0.098</td>
<td>0.088</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

*Significant partial correlation*
Chapter V

Discussion

The major finding from this study is that there were no statistically significant baseline and post-intervention differences in plasma adiponectin concentrations between healthy, non-obese MA and NHW young adults. This study also showed that a 7-day diet-exercise intervention that improves insulin sensitivity appears to do so independent of changes in circulating adiponectin concentrations. Our results are in disagreement with other studies in which exercise\textsuperscript{84, 86}, diet\textsuperscript{9} or a combined diet and exercise intervention\textsuperscript{85, 90} have resulted in significant increased plasma adiponectin concentrations with\textsuperscript{9, 86, 90} or without weight loss\textsuperscript{84}. These studies had different duration periods ranging from 1 week, similar to our study design, to up to two years. It is important to note that subjects in our study were non-obese compared to the obese characteristic of the subjects that participated in the studies cited above. This may suggest a difference response in plasma adiponectin concentrations with respect to a lifestyle intervention in obese vs. non-obese individuals.

Our study results are supported by those of Hulver et. al\textsuperscript{87}. This group of scientists reported significant insulin sensitivity improvements despite no significant difference in adiponectin concentration in sedentary healthy male and female subjects after a 6 month endurance exercise program. In our study, insulin resistance significantly improved pre-post intervention. Both markers of insulin resistance measured, HOMA-IR and Ins AUC, proportionally and significantly decreased in the two ethnic groups studied. This data

36
suggest that the beneficial effects of a lifestyle intervention on insulin sensitivity occur independently of changes in plasma adiponectin concentrations in both short (1 week) and long term (6 months) study designs. Despite these findings, several studies have reported an inverse association between plasma adiponectin levels and insulin resistance\textsuperscript{7, 9, 84-85}. Correlation analysis of baseline plasma adiponectin concentration and pre HOMA-IR and Ins-AUC are in agreement with this inverse association. Significant negative correlations were found between pre-plasma adiponectin and pre-HOMA-IR and pre-Ins AUC. It is important to note that the strength of these significant relationships decreased to a non-significant level when a partial correlation analysis controlling for gender and ethnicity was performed. This indicates that gender and ethnicity play a role in the association of plasma adiponectin and insulin resistance. Further study is necessary to help explain the role of gender and ethnicity in this association.

This study results also indicated that ethnicity did not have a significant main effect on plasma adiponectin concentrations pre-post intervention although NHW plasma adiponectin levels decreased around 7% post intervention while MA adiponectin concentrations had a smaller (~2%) non-significant decrease in plasma levels. Possibly in regard to plasma adiponectin concentration, these two ethnic groups respond differently to a combined diet and exercise intervention, but the study was underpowered to detect statistically significant differences. Further study is needed to clarify the association between ethnicity and change in plasma adiponectin concentrations in response to a lifestyle intervention.

Diet and exercise induced weight loss\textsuperscript{9, 86, 90} has been associated with increased plasma adiponectin concentrations. In our study, despite efforts from the study
researchers to maintain body weight unchanged for the duration of the study and eliminate body weight changes as a potential confounder variable, unexpected significant body weight pre-post intervention was observed. However, the change in body weight was not associated with changes in either insulin sensitivity (IAUC or HOMA-IR) or plasma adiponectin concentrations.

A positive and strong significant correlation and partial correlation after controlling for gender and ethnicity between pre-plasma adiponectin concentrations and pre-HDL cholesterol provides support to the notion that plasma adiponectin levels are predictive of HDL cholesterol concentrations. It remains to be seen whether or not this association is causal and if so, which precedes the other. Possibly therapeutic approaches to increase plasma adiponectin concentration levels may therefore contribute to the treatment of dyslipidemia.

**Strengths**

Ethnicity, a critical independent variable in our study, was traced back to at least 3 grandparents being either from Mexico or of European Caucasian descent. Both components of the lifestyle intervention, diet and exercise, were well supervised. All exercise sessions were monitored and all foods to be consumed for the duration of the intervention were provided to participants. Adherence to diet at home is difficult to determine, however, the subjects’ positive feedback regarding the quality, quantity, variety and taste of food provides some assurance that they complied with the dietary protocol. Nevertheless, the greater satiety owing to higher protein and fiber intakes may have contributed to the modest weight loss that occurred across both ethnic groups.
Limitations/Caveats

There were also some limitations in the design and implementation of this study. As mentioned before, statistical analysis of data may have been more powerful if sample size was bigger. This may have contributed to detect true differences between ethnic groups pre-post intervention. Also, a combined diet and exercise intervention design makes it unfeasible to assess independent contributions of diet or exercise alone on plasma adiponectin concentrations. Despite supervised exercise sessions and continuous follow up on participant’s adherence to the intervention diet an unexpected significant weight loss was observed. Body weight was a variable that we wanted to maintain constant throughout the duration of the study in order to eliminate it as a possible confounder variable but this goal was not achieved. The duration of the intervention phase may have not been long enough in order to observe changes in plasma adiponectin concentrations in these two ethnic groups. Longer intervention periods need to be evaluated to assess the possible effect of intervention duration on plasma adiponectin concentrations. In our study, the gold standard for directly measuring insulin sensitivity known as the hyperinsulinemic euglycemic glucose clamp\textsuperscript{109} was not used. Instead, insulin sensitivity was indirectly measured by calculating the IAUC and glucose AUC from the frequent blood samples obtained during the IVGTT. The major limitation of IAUC is that it does not provide accurate information on beta cell insulin secretion since only post-hepatic insulin delivery is considered and hepatic insulin extraction is not accounted for.\textsuperscript{110} However a decrease in circulating insulin concentration pre-post intervention combined with no significant changes in plasma glucose concentration and
assuming that hepatic extraction was constant at both time periods, is indicative of improvements in insulin sensitivity.

In summary, we can conclude the following from our study: 1) plasma adiponectin concentrations at baseline did not differ between ethnicities. 2) Baseline plasma adiponectin concentrations were significantly associated with insulin sensitivity across the entire sample; 3.) A one-week diet and exercise lifestyle intervention significantly improved insulin sensitivity in both MA and NHW adults, but changes in circulating adiponectin were not observed. Thus the improvements in insulin sensitivity resulting from a 7-day diet-exercise intervention did not result from improvements in circulating adiponectin. More research to elucidate plasma adiponectin ethnic differences and the effects of environmental factors such as exercise and a healthy diet on the association between plasma adiponectin concentrations and the development of insulin resistance and T2DM is needed.
REFERENCES


15. Hanley A, Bowden DW, Wagenknecht LE, et al. Associations of Adiponectin with Body Fat Distribution and Insulin Sensitivity in Nondiabetic Hispanics and


APPENDIX A: INFORMED CONSENT

COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

TITLE OF PROJECT: Interaction of Diet and Exercise on Chronic Disease Risk

NAME OF PRINCIPAL INVESTIGATOR: Chris Melby, Dr.P.H.

NAME OF CO-INVESTIGATORS: Matt Hickey, Ph.D., Stacy Schmidt, MS

CONTACT NAME AND PHONE NUMBER FOR QUESTIONS/PROBLEMS: Chris Melby, 491-6736, Matthew Hickey, 491-5727

SPONSOR OF THE PROJECT: Colorado Agricultural Experiment Station

PURPOSE OF THE RESEARCH: The purpose of the present study is to determine if your ethnic background influences how participating in a one-week diet and exercise program affects your risk for diabetes and other chronic diseases.

PROCEDURES/METHODS TO BE USED: After completing initial screening tests, eligible participants will be asked to spend 7 days during a baseline period consuming your usual diet and not participating in any formal exercise. You will then be asked to complete a 7-day period in which you exercise for 6 of the 7 days. During this second week, you will also eat food that we provide for you. If you are eligible to be in the study, your participation will require a time commitment of about 20 hours during a 19 day-period. There are a number of “exclusion criteria” (things that will make you ineligible for the study such as having diagnosed diabetes, certain types of medication, etc). Should you meet any of these exclusion criteria during the screening period, we will fully inform you as to the reason you can’t be in the study.

Depending on your eligibility for the study, your participation will involve a 19-day period:

DAYS 1-3: INITIAL SCREENING - During one of the initial days of the study, you will complete the following screening tests to help us determine if you are eligible to go the next phases. These tests will require about 90 minutes of your time.

Health and Medical History Questionnaire: You will need to answer questions about your medical history and personal health habits. Time required: 15 minutes

Ethnicity Questionnaire: You will need to identify your ethnic background. Time: 5 minutes

Food Preferences Questionnaire: You will need to answer questions about foods you like and don’t like. You will also be asked to list any food allergies you think you might have. Time: 10 minutes

Eating Disorders Questionnaire: You will be asked to complete a form that screens for eating disorders. Time: 5 minutes

Exercise Questionnaire: You will need to answer questions about your exercise habits. Time: 10 minutes
**Pregnancy test**- All women in the study will be asked to take a pregnancy test. If the pregnancy test is positive, you cannot be in this study. It is important that you do not become pregnant during the study. This will stop you from continuing the study. Time 10 minutes

**Body measurements**- Your height will be measured without you wearing shoes. Body weight will be measured on a normal scale. This will include the weight of light indoor clothing minus shoes. Your waist and hip circumference will be measured using a measuring tape. Time: 10 minutes.

**Blood pressure and blood glucose tests**- Following a 12-h fast (no food or beverages except water for 12 hours) you will have your blood pressure taken using normal procedures while you sit quietly in a comfortable chair. You will then have a small amount of blood taken from your fingertip (one drop). From this we will measure your blood sugar levels. If your blood pressure is greater than 140/90 or your blood sugar level higher than 126 mg/dl, you will not be able to participate in the study and you will be told to see your doctor to check for high blood pressure or diabetes. Time: 10 minutes.

**DAYS 4-11: BASELINE CONTROL PERIOD**- If your screening tests tell us you are eligible for the study, you will then begin a series of additional tests. During this time you will consume your typical diet. We ask that you do not participate in any exercise program during these 8 days. These tests will require a total of about 7 hours of your time.

**Body composition (fat and lean tissue)**- This will be performed using a machine called a dual energy X-ray absorptiometer (DEXA). This unit uses 2 low energy X-rays to determine the amount of body fat you have. You will be exposed to some radiation. But, the amount of radiation exposure in this procedure is very low, about 1/1,000 of the normal radiation exposure you receive yearly from what is called “background” radiation from the environment. Put another way, the exposure from a DEXA scan is less than the normal exposure in a flight from Denver to Chicago, and about 1/40th the exposure from a normal stomach X-ray you might receive at a hospital. This test will be performed in room 124 in the Human Performance Clinical Research Laboratory (HPCRL) located near Moby Gym. You will be asked to lie quietly on a bed in shorts and a T-shirt for about 15 minutes while the scan is performed. Time: 30 minutes.

**Resting Metabolic Rate**- This test involves reporting to the HPCRL or to room 216 Gifford between 7:00 and 9:30 am after a 12-hour overnight fast. You will be asked to lay on a bed for 30 minutes with a plastic canopy over your head or fitted with a mouthpiece to breathe into. Tubes connected to the canopy or mouthpiece measure how much air you breathe in and out. This measures how many calories you are burning while at rest. Time: 30 minutes.

**Physical Fitness**: This test involves walking and/or jogging on a motorized treadmill or riding on a stationary bicycle. It will be conducted in either Gifford 216 or the HPCRL. The grade (steepness) and speed of the treadmill (or pedal tension, if on a bike) will gradually increase until you are no longer able to continue. You will be asked to breathe through a mouthpiece during this test so we can measure the amount of oxygen your body consumes. In addition, we will measure your heart rate (using a heart rate monitor, which is like a small elastic belt you wear around your chest) and your blood pressure (using a small cuff that fits around your upper arm). Time: 40 minutes.

**Food Intake Record**: You will be asked to record your food intake on 3 consecutive days during this period. Time: 15 minutes.
Step counter: You will be asked to wear a simple step counter and record your step counts for days 4-18.

Fasting Blood Sample and Insulin Sensitivity Test: On day 11 of the study, you will be asked to report to the Hartshorn Health Center following a 12 hour fast for a blood sample. This means you will come to the lab in the morning. You will not have eaten any food or drank any beverages except water during the previous 12 hours. You will lie on a bed A hollow needle/plastic tube will be put in your forearm (or back of your hand if your veins are better there). First, about 2 teaspoons of blood will be taken from the tube in your arm. We will later determine how much glucose, fats (like cholesterol), hormones, and specific proteins are in your blood.

After this blood sample has been taken, you will begin the insulin sensitivity test. This test is to estimate the ability of insulin to cause sugar to move from your blood into your cells. Sugar water will be put into your blood though the tube (catheter). Blood will be collected from the catheter 15 times over a three hour period. After blood is taken from the catheter in your arm each time, a small amount of sterile salt-water will be used to flush the catheter to keep your blood from clotting inside the tubing.

We will later analyze your blood’s insulin and sugar levels. The amount of blood taken at each sample is about 1/2 teaspoon. Altogether, we will take about 10 teaspoons of your blood. You will probably not feel any pain when the blood is collected from the catheter in your arm. If the way your body responds to insulin is not normal or if your blood sugar levels are not in the normal range, you will be able to see a physician if you want. Time: 3.5 hours. This procedure will be performed 2 times: once during the baseline control period at one following the diet/exercise period.

Muscle Biopsy: During the insulin sensitivity test, you will have one muscle biopsy before you receive the sugar water. The small tissue sample (biopsy) will be obtained from the vastus lateralis, which is a large muscle in your thigh. We will determine your muscle's level of some molecules that allow insulin to work. The procedure involves numbing the skin using lidocaine, an anesthetic similar to novacaine, which you may have received at the dentist. If you are allergic to novacaine or have had any reaction to novacaine from your dentist, you should notify Professor Melby or Professor Hickey immediately and should not participate in this study. After numbing the skin, a small incision (less than the width of a pencil) is made in the skin over the muscle. The biopsy is obtained using a sterile needle. The muscle sample obtained is generally ~ ½ the size of an eraser on the end of a pencil. It is not uncommon to experience some mild soreness in the muscle that lasts for about a day. You should NOT restrict your activity, although you should also not perform any unusual or extremely vigorous activity for a few days. You will be provided with written instructions regarding proper care of the incision, and a telephone contact should you have any questions. This procedure will be performed 2 times: once during the baseline control period at the beginning of the study and once at the end following your 7-day diet and exercise period. This means you will be asked to have a total of TWO muscle biopsies.

DAYS 12-18: A SEVEN-DAY PERIOD OF DIET AND EXERCISE: After the screening and baseline tests are done during the first two weeks, you will participate in a diet and exercise program for seven days. Your diet will consist of eating foods (meals, beverages, and snacks) that we give you. The food will be healthy (lower in saturated fat and sugar than the diets of most Americans). You are expected to eat enough so you do not lose weight. You also should not eat so much that you gain weight. You will be expected to participate to exercise 6 of the 7 days during this period. Specifically, you be asked to do the following for the entire 7 days: 1). eat all your meals and snacks from the food we prepare and
provide you during the week (breakfast, lunch, dinner, snacks); 2.) come to the Gifford Building at least 3 times during the week to pick up food and be weighed at each visit; 3.) meet with a research dietitian twice when you come to the Gifford Building to determine your satisfaction with the food provided. If you don’t like certain foods we give you, we will find other food items to give you that you do like. 4.) exercise on a stationery bike or treadmill 6 times during the 7-day period for 35-40 minutes per session. Trained personnel will monitor all exercise sessions. Your exercise program will be in the South College Gym, Moby Gym, or the Gifford Building on the CSU campus. Time: ~ 8 hours total for the week

<table>
<thead>
<tr>
<th></th>
<th>Days 1-3</th>
<th>Days 4-11</th>
<th>Days 12-18</th>
<th>Day 19</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP/ Glucose</td>
<td>X</td>
<td></td>
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<tr>
<td>Weight</td>
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<tr>
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<td>X</td>
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<tr>
<td>Exercise</td>
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<td></td>
<td>X</td>
<td></td>
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<tr>
<td>Special diet</td>
<td></td>
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<td>X</td>
<td></td>
</tr>
<tr>
<td>Muscle Biopsy</td>
<td>X-Day 11</td>
<td>X-Day 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td></td>
<td>X-Day 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>X-Day 11</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Time (hours)</td>
<td>1.5</td>
<td>7</td>
<td>8</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**Day 19: SECOND INSULIN SENSITIVITY TEST AND SET OF BIOPSIES:** After the one week of special diet and exercise you will have a second insulin sensitivity test and the last one of two muscle biopsies. Time: 3.5 hours

**STUDY TIMELINE:**

**TOTAL TIME COMMITMENT:** approximately 20 hours

**RETENTION OF BLOOD AND MUSCLE SAMPLES**
You should understand that we plan to keep any extra muscle and blood samples that are not used in the analysis for this study. In other words, if we have any “extra” blood or muscle we will keep them in a freezer in our lab. It is very possible that we will use all of the blood and muscle obtained in this study and will have none left, but in the event that we do, we would like your permission to keep the samples in the event that they can be used for further research. We will use these samples in the future solely for additional research on obesity and metabolism; specifically, all future research will simply be an extension of what we hope to accomplish with the current study. We may simply analyze your blood for the presence of other hormones or metabolites, or analyze your muscle for other enzymes, etc. We have NO plans to store DNA in this study. Your stored samples will be coded in such a way that your confidentiality will be maintained. Only the Principal Investigators (Professors Melby and Hickey) will have access to the coding system for your samples. There is a possibility that your samples may be shipped to other departments on the CSU campus, or to colleagues at other Universities for assistance with analysis. Under such circumstances, the same coding system will be used, so researchers in other labs will not be able to identify you. We do not anticipate ANY commercial product development from your tissue, the samples will be used solely for research purposes. You should be advised that we do NOT have plans to recontact you in the future regarding any additional analyses, but will seek full approval of the CSU Regulatory Compliance Office prior to initiating any further research on your samples.
By checking “Yes” below and signing on the accompanying line, you are agreeing to allow the investigators retain any muscle and blood samples obtained during this study. If you do not wish the investigators to retain any samples, please check the box marked “No” and also sign on the accompanying line.

The investigators may keep any muscle or blood samples obtained during the course of this study for future research on obesity and metabolism

[ ] YES  [ ] NO

______________________________  ________________________
Signature                  Date

EXERCISE PROGRAM CONTINUATION (PHASE II)
After you have completed the 19-day study detailed above, you will be asked if you would like to continue with the exercise program for an additional 7 weeks. The exercise will consist of 35-40 minutes per session 4 sessions a week on a stationery bike or treadmill. Trained personnel will monitor all exercise sessions. Your exercise program will be in the South College Gym, Moby Gym, or the Gifford Building on the CSU campus. Should you choose to participate, you will not be provided with food but you are expected to eat enough so you do not lose weight. To monitor weight stability, you will be weighed the first exercise session of each week. If you choose to participate in the additional 7 weeks of exercise, you will not receive additional compensation.

Fasting Blood Sample: On day 68 of phase II (i.e., after completing the 7 additional weeks of exercise training), you will be asked to report to the Hartshorn Health Center following a 12 hour fast for a blood sample. This means you will come to the lab in the morning. You will not have eaten any food or drank any beverages except water during the previous 12 hours. You will lie on a bed. A hollow needle will be put in your forearm (or back of your hand if your veins are better there). Approximately 2 teaspoons of blood will be taken from the tube in your arm. We will later determine how much glucose, fats (like cholesterol), hormones, and specific proteins are in your blood.

Time: ~3 hours per week, total time ~21 hours

I would like to participate in an additional 7 weeks of supervised exercise following day 19 of the study.

______________________________  ________________________
Signature                  Date
PHASE II STUDY TIMELINE:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Days 1-3</th>
<th>Days 4-11</th>
<th>Days 12-18</th>
<th>Day 19</th>
<th>Day 20-68</th>
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<tr>
<td>Screening</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP/Glucose</td>
<td>X</td>
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</tr>
<tr>
<td>Weight</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
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</tr>
<tr>
<td>Diet Analysis</td>
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<td>X</td>
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<td></td>
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</tr>
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<td>X</td>
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<td>X</td>
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<tr>
<td>Exercise</td>
<td>X</td>
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<td></td>
<td>X</td>
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<tr>
<td>Special diet</td>
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<tr>
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<td>X-Day 11</td>
<td>X</td>
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<td>X-Day 11</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>X-Day 11</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Time (hours)</td>
<td>1.5</td>
<td>7</td>
<td>8</td>
<td>3.5</td>
<td>21</td>
</tr>
</tbody>
</table>

RISKS INHERENT IN THE PROCEDURES:

1). **DEXA**: The risks associated with the DEXA are very low. The radiation you will receive in this study is less than 1/3000th of the FDA limit for annual exposure. Put another way, you could receive 3000 DEXA scans in a single year and still not meet the FDA limit for radiation exposure. In this study, you will receive only a single scan. The more radiation you receive over the course of your life, the more is the risk of having cancerous tumors or causing changes in genes. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risks is not known. Women who are pregnant or could be pregnant should receive no unnecessary radiation and should not participate in this study.

2). **Blood Samples**: The risks associated with blood drawing include bruising, vein inflammation, slight risk of infection, local soreness, and fainting. These are all very minor risks and if present, are generally resolved in less than a day.

3). **Resting Metabolic Rate measurement**: There is no known risk associated with this procedure. You may experience some minor discomfort associated with this measurement if you have claustrophobia, but this is very unlikely. The canopy used is a large, see-through plastic bubble. There is adequate space and breathing is unrestricted, whether you are in the canopy or you use a mouthpiece.

4). **Cardiorespiratory Fitness and Exercise**: The exercise test is a standard test for determining the presence of heart and lung problems. 1 in 10,000 individuals with cardiovascular disease may die and 4 in 10,000 may have abnormal heart beats or chest pain. The exercise program will be less intense than your exercise test, so the risks are less. However, as with any exercise, there is the possibility of muscle soreness and muscle, bone, or joint injury.

5). **Insulin Sensitivity Test**: The risks of obtaining your blood from a tube put in your vein are similar to those that could occur from a blood draw. Any time a blood collection catheter is inserted, there is a small risk that you may faint, experience local soreness, bruising or infection. There is also a small risk of experiencing low blood sugar during the test with symptoms of headache, tremor, and dizziness.

6). **Muscle Biopsy**: The risks associated with the muscle biopsy include discomfort, localized soreness, bruising, infection, and minor scarring. The discomfort and localized soreness are
likely, but generally last only 24-48h. Temporary scarring is also expected, the natural course of wound healing varies substantially from individual to individual, but the scar will be less than ½ inch long, and is generally difficult to distinguish within 8-12 months after the biopsy. The risk of bruising is low, and infections are extremely rare.

7). Research Diets: There is a small risk that you could get a food-borne illness from the research diets. The food will be prepared under the supervision of a trained Chef. Meal preparation will occur in nutrition laboratories in the Department of Nutrition at CSU or in kitchens which supply meals to campus residence halls. All food preparation will be done in accordance with standard procedures designed to minimize the risk of illness. Thus, the likelihood of a food-borne illness is remote.

It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

BENEFITS:
You will receive detailed diet and body composition data, and information on the role of diet and exercise in maintenance of health. You will receive 7 days of food at no cost to you. If you choose to participate in the additional 7 weeks of exercise, you have the potential to benefit from increased physical fitness associated with regular exercise.

COMPENSATION
You will be paid $100 upon completion of this study, and an additional $25 for each of the 2 possible biopsies, for a total of $150 dollars possible. While the biopsies are an important part of the study, the information we obtain from other measures (i.e. insulin sensitivity) is important to us as well. You may still participate even if you choose not to have the biopsies, but your payment will be less. If you choose at any point not to continue the study, you will be paid $75 upon completion of ALL tests between days 1-11. If you choose to participate in the additional 7 weeks of exercise, you will not receive additional compensation.

REASONS WHY YOU MAY BE REMOVED FROM THE STUDY
As mentioned, we are aware that this study requires a significant time commitment from you as a volunteer. It is very important to the study that you not miss scheduled visits with study personnel. In the event that something comes up that will make you miss a visit, please call and let us know. Please also note that we may call you if a visit is missed, simply to check and make sure everything is OK. If you have conflicts that require you to miss more than 10% of your scheduled visits, we will have to remove you from the study. If this happens, we will contact you and let you know the reason why you will not be allowed to continue, and make arrangements to pay you for the portion(s) of the study you have completed. Should our testing reveal information that suggests you need to be referred for medical care, we will put you in contact with Dr. Russell Risma, M.D., at the Hartshorn Health Service at Colorado State University, who is the physician contact for this study.

CONFIDENTIALITY:
Your data will be coded and kept in a locked file cabinet on the CSU campus. A copy of the coded data may be sent to the sponsor of this project. A summary of the findings will be published in a science journal. However, you will not be identified in relation to your data at any point.

LIABILITY:
Because Colorado State University is a publicly-funded, state institution, it may have only limited legal responsibility for injuries as a result of participation in this study under a Colorado law known as the Colorado Government Immunity Act (Colorado Revised Statutes, section 24-10-101, et seq.). In addition, under Colorado law, you must file any claims against the University within 180 days after the date of the injury. In light of these laws, you are encouraged to evaluate your own health and disability insurance to determine whether you are covered for any injuries you might sustain by participating in this research, since it may be necessary for you to rely on individual coverage for any such injuries. If you sustain injuries which you believe were caused by Colorado State University or its employees, we advise you to consult an attorney. If you have any questions about your rights as a volunteer in this research, contact Janell Barker, IRB Administrator, (970) 491-1655 or janell.barker@research.colostate.edu

PARTICIPATION:

Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled. Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 7 pages.

________________________________________  _______________________________________
Participant Name (print)Date  Participant Signature

________________________________________  _________________________________
Investigator or co-investigator Signature  Date
APPENDIX B: ETHNICITY IDENTIFICATION FORM

PARTICIPANT ETHNICITY IDENTIFICATION FORM

Nutrition and metabolic Fitness Laboratory, Colorado State University

ID #________________  Date: __________________

1. Please identify your ethnicity: ______
   A. Mexican American  G. Other Spanish
   B. Mexican/Mexicano  H. Caucasian
   C. Puerto Rican      I.  Black
   D. Cuban            J. Asian/Pacific
   E. Other Latin American  Islander
   F. Native American

2. What are your parents’ surnames?
   Father: ____________________________
   Mother: ___________________________

3. What are your parents’ countries of origin?
   Father: ____________________________
   Mother: ___________________________

4. Please identify the ethnicity of your 4 grandparents:
   (Use the letters from Question #1)
   Father’s father: ________
   Father’s mother: ________
   Mother’s father: ________
   Mother’s mother: ________
Father’s mother: ________
Mother’s father: ________
Mother’s mother: _____

5. What is your primary (first) language spoken

_____________________
APPENDIX C: HEALTH HISTORY QUESTIONNAIRE

Colorado State University
CONFIDENTIAL HEALTH HISTORY QUESTIONNAIRE

Study______________  Date______________  Subject
ID______________

Reviewed by PI: ______________________

PLEASE PRINT

Current Age_______  Height_______
Weight_______

GENERAL MEDICAL HISTORY

Do you have any current medical conditions?  YES  NO  If Yes, please explain:

Have you had any major illnesses in the past?  YES  NO  If Yes, please explain:

Have you ever been hospitalized or had surgery?  YES  NO  If Yes, please explain:
(include date and type of surgery, if possible)

Have you ever had an electrocardiogram (EKG)?  YES  NO  If Yes, please explain:
(a test that measures your heart’s activity using an electrical tracing)
Are you currently taking any medications, including aspirin, hormone replacement therapy, or other over-the-counter medications?  

- [ ] YES  
- [ ] NO  
If yes, please explain:

<table>
<thead>
<tr>
<th>Medication</th>
<th>Reason</th>
<th>Times taken per Day</th>
<th>Taken for how long?</th>
</tr>
</thead>
</table>

PI Initials __________________________

Are you currently taking any nutritional supplements, such as Ginko, St. John’s Wort, or others?  

- [ ] YES  
- [ ] NO  
If Yes, please explain:

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Reason</th>
<th>Times taken per Day</th>
<th>Taken for how long?</th>
</tr>
</thead>
</table>

Have you been diagnosed with diabetes?  

- [ ] YES  
- [ ] NO  
If yes, please explain:

Age at diagnosis ________

Have you been diagnosed with a thyroid disorder?  

- [ ] YES  
- [ ] NO  
If yes, please explain:

FAMILY HISTORY  
Please indicate the current status of your immediate family members.

<table>
<thead>
<tr>
<th>Family Member</th>
<th>Age (if alive)</th>
<th>Age of Death</th>
<th>Cause of Death</th>
</tr>
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<tbody>
<tr>
<td>Father</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>Mother</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>Brothers/Sisters</td>
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<td>_______</td>
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</table>
Do you have a family history of any of the following: (Blood relatives only, please give age at diagnosis if possible)

<table>
<thead>
<tr>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>a. High Blood Pressure</td>
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</tr>
<tr>
<td>b. Heart Attack</td>
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<tr>
<td>c. Coronary bypass surgery</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td>d. Angioplasty</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>e. Stroke</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td>f. Diabetes</td>
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<td>☐</td>
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<tr>
<td>g. Obesity</td>
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<tr>
<td>h. Other (Please List)</td>
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Relation     Age at Diagnosis

PI Initials_______________________

MUSCULOSKELETAL HISTORY

<table>
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<th>NO</th>
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<td>Any current muscle injury or illness?</td>
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</tr>
<tr>
<td>Any muscle injuries in the past?</td>
<td>☐</td>
</tr>
<tr>
<td>Muscle pain at rest?</td>
<td>☐</td>
</tr>
<tr>
<td>Muscle pain on exertion?</td>
<td>☐</td>
</tr>
<tr>
<td>Any current bone of joint (including spinal) injuries?</td>
<td>☐</td>
</tr>
<tr>
<td>Any previous bone or joint (including spinal) injuries?</td>
<td>☐</td>
</tr>
<tr>
<td>Painful joints?</td>
<td>☐</td>
</tr>
<tr>
<td>Swollen joints?</td>
<td>☐</td>
</tr>
<tr>
<td>Edema (fluid build up)?</td>
<td>☐</td>
</tr>
<tr>
<td>Pain in your legs when you walk?</td>
<td>☐</td>
</tr>
</tbody>
</table>

If you checked YES to any of the above, you will be asked to clarify your response by an investigator so we can be sure to safely determine your ability to participate

NEUROLOGICAL HISTORY

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of seizures</td>
<td>☐</td>
</tr>
<tr>
<td>Diagnosis of epilepsy</td>
<td>☐</td>
</tr>
<tr>
<td>History of fainting</td>
<td>☐</td>
</tr>
</tbody>
</table>

GASTROINTESTINAL HISTORY

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of ulcers</td>
<td>☐</td>
</tr>
<tr>
<td>History of colitis</td>
<td>☐</td>
</tr>
<tr>
<td>History of chronic diarrhea</td>
<td>☐</td>
</tr>
</tbody>
</table>
History of chronic constipation □ □

**REPRODUCTIVE HISTORY**

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Currently pregnant</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Think you might be pregnant</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Planning on becoming pregnant in the near future</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Currently using Oral Contraceptives</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>History of Menstrual cycle irregularities</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

PI Initials_______________________

**TOBACCO HISTORY** (check any that apply)

<table>
<thead>
<tr>
<th>Tobacco Use</th>
<th># per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Cigarette</td>
<td></td>
</tr>
<tr>
<td>Cigar</td>
<td></td>
</tr>
<tr>
<td>Pipe</td>
<td></td>
</tr>
<tr>
<td>Chew Tobacco</td>
<td></td>
</tr>
<tr>
<td>Snuff</td>
<td></td>
</tr>
</tbody>
</table>

**CARDIORESPIRATORY HISTORY**

<table>
<thead>
<tr>
<th>Condition</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presently diagnosed with heart disease</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>History of heart disease</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Heart murmur</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Occasional chest pain or pressure</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Chest pain or pressure on exertion</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Heart valve problem</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Abnormal heart rhythm</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Edema (fluid build up)</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>High Cholesterol</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>History of rheumatic fever</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Episodes of fainting</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Daily coughing</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>
Shortness of breath
   At rest
   Lying down
   After 2 flights of stairs
Asthma
Emphysema
Bronchitis
History of bleeding disorders
History of problems with blood clotting

If you checked YES to any of the above, you will be asked to clarify your response to an investigator so we can be sure to safely determine your ability to participate.

PI Initials______________________

**DIET HISTORY**

Have you ever dieted? YES NO
If YES, have you dieted within the past 12 months or are you currently on a diet? YES NO

If you have dieted within the past 12 months, please describe the diet:

   a). Name (if applicable): ________________________________

   b). Prescribed by a Physician/nutritionist YES NO
   
   c). Have you lost weight? YES NO
   
   d). Duration of the diet? ______________

What was your weight 12 months ago? ______________

What was your weight 6 months ago? ______________

Have you dieted other than in the past 12 months? YES NO

If YES, please answer the following:

   a). How many times have you dieted?
   
   b). How old were you?
   
   C). Weight loss (amount)? YES NO

History of eating disorders? YES NO
EXERCISE HISTORY

How many times a week do you participate in moderate to high intensity exercise? (examples include jogging, biking, aerobics, basketball, swimming, etc.)

How long do these exercise sessions last?

You may be asked to complete a more detailed diet survey if you are volunteering for a research study.