RELATIONSHIPS BETWEEN DIET, WEIGHT LOSS, INSULIN RESISTANCE AND ADIPONECTIN LEVELS AMONG OVERWEIGHT/OBESE ADULTS

by

Sushama Dhakal Acharya

BSc(Hons), University of Wollongong, Australia, 1995

MS, University of Hawaii, 2001

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This dissertation was presented

by

Sushama Dhakal Acharya

It was defended on

December 6, 2010

and approved by

Dissertation Advisor:
Lora E. Burke, PhD, MPH
Professor, Department of Health and Community System, School of Nursing, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh

Committee Member:
Maria M. Brooks, PhD
Associate Professor, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh

Committee Member:
Rhobert W. Evans, PhD
Associate Professor, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh

Committee Member:
Faina Linkov, PhD
Assistant Professor, Department of Medicine, Division of Hematology and Oncology, School of Medicine, University of Pittsburgh

Lora E. Burke, PhD, MPH

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Sushama Dhakal Acharya, PhD

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Adiponectin has been shown to improve insulin sensitivity, regulate glucose and lipid metabolism and exert anti-atherosclerotic effects. This dissertation, designed as three research papers, aimed to examine relationships between diet, weight loss, insulin resistance and adiponectin levels among overweight/obese adults who were participating in a behavioral intervention for weight loss. Data from the ancillary study to the PREFER trial were used for two of the papers and secondary data analysis from the SMART trial was conducted for the third paper. Both parent studies were randomized clinical trials that included a behavioral intervention for weight loss. The first study compared the effect of a standard calorie- and fat-restricted diet and a calorie- and fat-restricted lacto-ovo-vegetarian diet on changes in adiponectin levels at six months (N=143). Weight loss was associated with increased total (β(s.e) = -0.71(0.27); P = 0.003) and high molecular weight (HMW) adiponectin levels (β(s.e) = -1.37(0.47); P = 0.001); however, they were independent of the diet type. The second study examined whether baseline levels or intervention-associated changes in adiponectin levels were associated with insulin resistance after six months (N=143). At baseline, we found significant inverse associations between total (β(s.e) = -0.26(0.05); P < 0.001) and HMW(β(s.e) = -0.38(0.09); P < 0.001) adiponectin levels and homeostasis model assessment of insulin resistance (HOMA) independent of weight. At six months, there was a significant inverse association between changes in total adiponectin and HOMA (β(s.e) = -

0.17(0.08); P = 0.04) that was independent of baseline weight and weight loss. However, the association between changes in HMW adiponectin and HOMA was not significant. The third study assessed the longitudinal relationships of weight, waist circumference and body composition with adiponectin levels after six and 12 months (N=133). A significant increase in adiponectin was observed with significant reductions in weight, body mass index, waist circumference, and percent body fat (P for all, < 0.001). Our findings provide evidence for the importance of weight loss as a significant public health preventive measure to enhance adiponectin levels among the studied population, which could impact the progression of atherosclerosis and subsequent cardiovascular disease.

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1.0 DISSERTATION OBJECTIVES

Adipose tissue is no longer considered an inert tissue for storing energy but is now recognized as an active endocrine organ secreting adipokines, cytokines and a diverse range of inflammatory markers[1]. Adiponectin is one of the adipokines secreted by white adipose tissue and has been suggested to improve insulin sensitivity, regulate glucose and lipid metabolism and might play a role in the development of diabetes and atherosclerosis[2-4]. Previous studies suggest that adiponectin levels tend to decrease with an increasing body weight and a larger increase in adiponectin may occur with a greater weight loss[2, 5, 6].

A lower level of adiponectin in male patients was found to correlate significantly and independently with coronary heart disease (CHD)[4]. Subjects with CHD had lower adiponectin levels compared with age and body mass index (BMI) adjusted controls suggesting that adiponectin confers a protective effect against atherosclerosis[7, 8]. Significant negative correlations have been described between plasma adiponectin levels and BMI, percent body fat, waist-to-hip ratio (WHR) and intra-abdominal fat in adults[2]. In persons with diabetes, plasma adiponectin concentrations were lower in age and BMI-matched non-diabetic men and women[3]. Individuals with lower adiponectin concentrations were found to be at higher risk of developing type 2 diabetes than those with higher concentrations suggesting a potential role of adiponectin in the pathogenesis of type 2 diabetes[9]. In addition, decreased circulating adiponectin levels in obesity were associated with higher blood pressure and an increased risk of CHD[10, 11]. Moreover,

lower adiponectin levels were linked to future coronary events in men with type 2 diabetes[12]. Furthermore, low plasma adiponectin concentration appeared to be an independent predictor of all cause mortality, cardiac mortality and myocardial infarction in patients presenting with chest pain[13]. At a cellular level, adiponectin has been found to suppress inflammation of the vascular wall[14], suppress the proliferation of vascular smooth muscle cells[15] and reduce foam cell formation[15]. The anti-inflammatory properties of adiponectin demonstrated a reciprocal association between adiponectin and C-reactive protein in both the blood stream and adipose tissue[4].

Results of these studies highlight the importance of exploring potential preventive approaches to improve adiponectin levels. There is evidence that a modest intentional weight loss through lifestyle changes is an effective means for preventing obesity-associated disorders. However, intervention studies focusing on changes in adiponectin level in humans have shown mixed results. Thus, the purpose of this dissertation was to better understand the relationship of diet, weight loss and insulin with adiponectin. Following are the aims for the dissertation:

1. Compare the effect of a standard calorie-restricted, low-fat diet (STD-D) vs. a lacto-ovo-vegetarian diet (LOV-D) on adiponectin levels at six months during a 12-month lifestyle intervention intended to achieve weight reduction.
2. Determine whether baseline or intervention-associated changes in adiponectin levels were associated with insulin resistance after six months of behavioral treatment for weight loss.
3. Assess the longitudinal relationships between weight, waist circumference, body composition and adiponectin levels at six and 12 months of a behavioral intervention for weight loss.

2.0 BACKGROUND

2.1 ADIPOSE TISSUE

Adipose tissue is no longer considered an inert tissue mainly devoted to energy storage but is now recognized as an active endocrine organ secreting several hormones and a diverse range of other protein factors[16]. In general, adipose tissue can be divided into two major types: white adipose tissue (WAT) and brown adipose tissue. WAT is composed of mainly adipocytes and other distinct cell types including mature adipocytes, pre-adipocytes, fibroblasts and macrophages, all of which participate, to a greater or lesser extent in WAT secretary functions[1]. A number of proteins secreted by adipocytes are called adipocytokines. Leptin, adiponectin, vistafin, and resistin are a few of the well known adipocytokines. Macrophages in WAT also secrete a number of cytokines including interleukin-6 (IL-6), tumour necrosis factor alpha (TNFά), interleukine-5 (IL-5), macrophage chemoattractant protein (MCP). All hormones and protein molecules secreted by adipose tissue are collectively called adipokines. The number and range of adiposity secretary proteins is continuing to expand rapidly and approximately 50 different molecular entities have been identified so far[16].

2.1.1 Adiponectin

Adiponectin, also known as adipoQ, Acrp30, apM1 and GBP28, is a protein molecule that is secreted by the adipocytes of WAT. There is a tendency for reduced adiponectin levels in obese individuals and increased levels in those with anorexia nervosa[17, 18]. Other studies described a significant negative correlation between BMI and plasma adiponectin levels in both men and women and that adiponectin levels are negatively correlated with percent body fat, waist–to-hip ratio and intra-abdominal fat[2, 6]. The secretion of adiponectin into the bloodstream is not regulated by subcutaneous but rather by visceral adipose tissue[19].

Furthermore, plasma adiponectin concentrations are reported to increase following weight loss[3, 20]. In patients with diabetes, plasma concentrations are lower in age and BMI-matched non-diabetic men and women[3]. Adiponectin concentrations correlate negatively with fasting plasma insulin concentrations and 2-hr glucose concentrations in glucose tolerance test and correlate positively with insulin sensitivity[2, 6]. In examining lipid profiles, serum triglycerides levels correlated negatively and high density lipoprotein cholesterol levels correlated positively with plasma adiponectin levels[3]. In a study with Pima Indians, individuals with high adiponectin concentrations were found to be at lower risk of developing type 2 diabetes than those with low concentrations suggesting a potential role of adiponectin in the pathogenesis of type 2 diabetes[9].

The normal plasma level for adiponectin in healthy humans is 1.9-17.0 mg/dl with significantly lower levels reported in obese subjects[17]. Hypoadiponectinemia, a plasma adiponectin level of < 4.0 ug/ml, in males was found to correlate significantly and independently with coronary artery disease (CAD)[4]. Individuals with CAD have lower adiponectin levels

compared with age and BMI adjusted controls[8] suggesting that adiponectin, in contrast to other adipokines, confers a protective effect against atherosclerosis. Also, decreased circulating adiponectin levels in obesity are associated with an increased risk of CAD[10, 11]. Additionally, adiponectin is linked to future coronary events in men with type 2 diabetes[12]. Low plasma concentration is an independent predictor of all cause mortality, cardiac mortality and myocardial infarction in patients presenting with chest pain[13].

Adiponectin reduces the production of TNF-ά[21] and inhibits IL-6 production[22]. Adiponectin and TNF-ά mutually inhibit each other's production in adipose tissue and adiponectin can counteract the pro-inflammatory effects of TNF-ά in vascular cells[23]. Since IL-6 induces the release of hepatic VRP, adiponectin may indirectly inhibit IL-6 and CRP expression. It also suppresses the transformation of macrophages to foam cells[17].

It has been reported that circulating adiponectin is present in three distinct forms; low molecular weight (LMW), medium molecular weight (MMW) and high molecular weight (HMW) form[24]. Although the precise biological activities of these forms are not well understood, it has been suggested that HMW adiponectin is the most active form of adiponectin[25]. HMW adiponectin has been reported to be an important factor in explaining the metabolic syndrome[26, 27] and is more closely associated with the insulin resistance syndrome than total plasma adiponectin level[25, 26]. Also, HMW adiponectin has been reported to confer a protective effect against coronary artery disease[28]. However, many intervention studies that included a low-calorie diet that induced weight loss have been limited by the measurement of only total adiponectin. Hence, little is known about the changes in the HMW level relative to the total adiponectin level induced by such interventions.

In summary, adiponectin levels are decreased in obesity and are inversely correlated with insulin resistant states in obesity. Adiponectin has been shown to inhibit the TNF-ά induced changes in monocyte adhesion molecule expression and in the endothelial inflammatory response[8]. Adiponectin concentrations were negatively correlated with WHR, independent of gender and overall adiposity[19]. Taken together, adiponectin appears to act as an anti-inflammatory molecule with some protection against diabetes and atherosclerosis [29].

2.1.2 Obesity and Inflammation

Obesity, particularly visceral adiposity, is associated with chronic low-grade inflammation, as indicated by an increased level of inflammatory markers in the circulation of obese subjects. The WAT of obese individuals contains an increased number of macrophages compared with that of lean persons with a possibility of higher secretion of pro-inflammatory molecules. It has been hypothesized that adipokines, cytokines and other factors produced and secreted by WAT are responsible for the chronic inflammatory state of visceral obesity. The combination of inflammation, hypertension and dyslipidemia increases the likelihood of development of type 2 diabetes and CVD[30]. However, the ultimate reason for increased production of pro-inflammatory factors by WAT in obesity has not been identified[31].

One of the key developments in obesity research over the past four years is the recognition that the disorder is characterized by chronic mild inflammation[30]. The main basis for this view is that there is an increased circulating level of inflammatory markers in the obese individuals. Given the observation that WAT expresses and secretes a number of inflammation-related proteins, it is probably that it is a major source of the increase in inflammatory markers in obesity thereby increasing risks for the development of diabetes and CVD.

On the basis of available evidence, it could be hypothesized that the increased release of pro-inflammatory adipokines, mainly IL-6 and TNF-ά from the visceral adipose tissue along with reduced secretion of anti-inflammatory adipokines and cytokines, mainly adiponectin and IL-10 can generate a low grade chronic inflammatory state. This imbalance of pro-inflammatory and anti-inflammatory adipokines might play a role in the future development of diabetes and atherosclerosis through both a reduced insulin sensitivity and endothelial dysfunction. Figure 1 depicts a proposed mechanism linking adiposity and adiponectin with chronic diseases, such as atherosclerosis and diabetes.

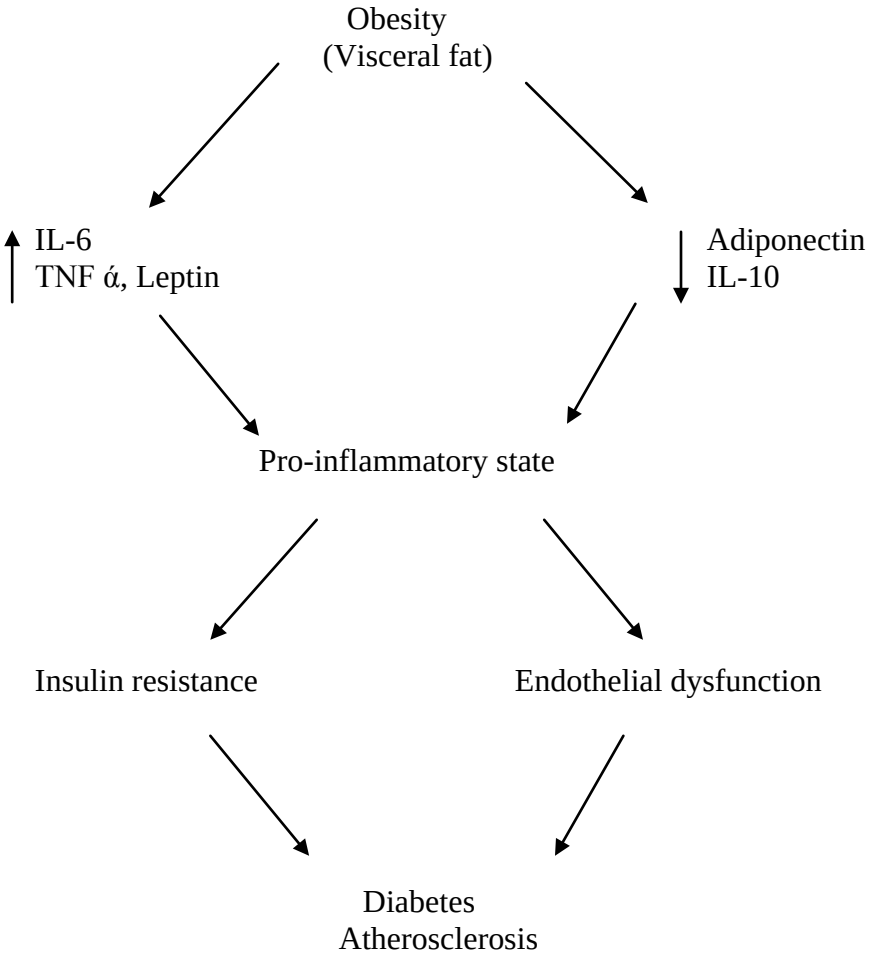


Figure 2-1 Proposed link among adiposity, adiponectin and diabetes or atherosclerosis

2.2 ADIPONECTIN AND DIET

Epidemiological studies have suggested that increased adiponectin levels are associated with consuming deep yellow vegetables[32], a Mediterranean-type diet[33], moderate alcohol[34], having a high score for the dietary pattern characterized by a high intake of fresh fruits[35] and adherence to a healthy dietary pattern as measured by an alternate healthy eating index[36]. A dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products and low consumption of refined cereals was positively associated with adiponectin levels in healthy women[37]. Observational studies have revealed significant negative associations between higher energy intake, lower physical activity and adiponectin levels among women[38] and men[32].

However, short-term controlled studies examining the effect of a very-low calorie diet (VLCD) on adiponectin levels in small groups of adults have shown conflicting results. Anderlova et al.[39] did not find significant changes in adiponectin levels in obese women after a VLCD (550 cal/day) for three weeks. Additionally, obese women consuming a liquid diet of 800 cal/day for four weeks did not significantly increase adiponectin levels[40]. Xydakis et al.[41] examined the impact of a VLCD (600-800 cal/day) on adiponectin in obese adults with and without the metabolic syndrome (MS) for 6-8 week duration and found no change in adiponectin levels in both groups. However, in a similar study between subjects with and without the MS, adiponectin levels in both groups increased after administration of a VLCD (450 cal/day) for 16 weeks followed by two weeks of re-feeding[42]. The possible explanation for the inconsistent findings of these studies might be variations in type and duration of the VLCD and also the populations studied. The generalizability of these findings may be limited due to the severe restrictiveness of the VLCD. In order to consider the beneficial effects of diet on adiponectin for an intervention, it is essential to examine the relationship between adiponectin and a diet that individuals can follow for the long term.

Although a hypocaloric diet induced weight loss and usually improved co-morbidities associated with obesity in several descriptive studies, the circulating adiponectin levels remained either unchanged[43], increased[44-46] or even decreased[47]. Dvarakova-Lorenxova et al.[43] reported no change in adiponectin levels after nine weeks of dietary education and supervised physical activity in 90 obese women, but limited detail was provided regarding the type of diet. In contrast, a 12-week, low caloric, low-fat diet among 20 overweight/obese premenopausal women resulted in a 36% increase of adiponectin levels[46]. Similarly, Brunn et al.[45] reported increased adiponectin levels following a 15-week lifestyle intervention among 23 severely obese subjects. Increased adiponectin levels were observed among 21 obese children while receiving a psycho-educational intervention suggesting that adiponectin in obese children can be normalized[44].Similar improvements in adiponectin levels were reported in104 overweight and obese children after a 12-month lifestyle intervention[48]. Conversely, decreased adiponectin levels were found among 15 healthy, normal-weight women after following a 4-week low caloric diet[47]. In summary, these studies reported inconsistent findings and had several limitations, in particular a small sample and a brief intervention period. Thus, the evidence provided by these studies is inconclusive about the effect of diet on adiponectin levels.

Results of randomized clinical trials have also been inconsistent regarding the effects of dietary factors on adiponectin levels. No changes were observed in adiponectin following either a moderate fat/moderate carbohydrate or low fat/high carbohydrate diet in obese women for 10 weeks[49]. Similarly, Cardillo et al.[50], deLeu et al.[51] and Keogh et al.[52] found no difference in adiponectin levels between the two groups of obese individuals that were randomly assigned to either a low carbohydrate or a low fat diet for a period of 12 months, 36 months or 12 weeks, respectively. Additionally, no significant changes were found in adiponectin levels in the lifestyle intervention group when compared with insulin treatment and combined treatment of insulin and lifestyle intervention in adults with type 2 diabetes for one year[53]. A marginal increase in adiponectin levels

was observed in the diet and exercise intervention group when compared with the control groups in obese women after 14 weeks[54] and non obese healthy women after 12 months[55].

No differences in adiponectin were observed between the lifestyle intervention and the control groups in obese adolescents over a one-year period[56]. Compared with the control group, a significant increase in adiponectin was observed in the group following a lifestyle intervention that included a Mediterranean-style diet[57]. A moderately low-fat diet intervention produced favorable changes in adiponectin levels in comparison with the weight maintenance intervention over a 16-week intervention[58]. Similarly, a one-year hypocaloric dietary intervention had a significant positive effect on adiponectin levels compared with the exercise, the diet and exercise and the control groups[59]. A significant improvement in adiponectin levels was observed at three months in the fiber supplementation group when compared with the placebo group, suggesting a possible role of specific dietary factors on adiponectin[60].

There is ample evidence that following a vegetarian diet may be beneficial to general and cardiovascular health. This eating pattern has been linked to less weight gain[61], improved lipid profile[62] and increased body leanness[63] compared to those following a carnivorous diet. There is substantial evidence that the amount and type of foods eaten have profound effects on risk factors for CHD (lipid, glucose, insulin). Thus, it is plausible that adiponectin also can be significantly affected by changes in dietary composition. An understanding of adiponectin physiology in response to various diets could be of value in identifying a more beneficial diet to prevent or reduce the inflammatory state that is associated with the development of diabetes and CHD.

In summary, the effect of diet on serum adiponectin level varies depending on the type of diet consumed, the study population, and duration of the dietary intervention. Although inconclusive, many studies suggest that adiponectin levels might be modified by dietary intake. What remains

unknown is the effect of a LOV-D on adiponectin levels in healthy overweight/obese adults who are participating in a behavioral and dietary intervention for weight loss over a longer period.

2.3 ADIPONECTIN AND BODY WEIGHT

Several studies have reported decreased adiponectin levels in obese individuals compared with normal weight individuals[17, 20, 64]. Significant negative correlations have been described between adiponectin levels and BMI, percent body fat, WHR and intra-abdominal fat in adults[2, 6, 65]. The Cardia Study showed decreased adiponectin levels with increasing weight and intra-abdominal fat during five years of observation[66]. Weight loss has been found to increase adiponectin concentration[3, 20, 67] and there is a tendency for increased levels to be present in those with anorexia nervosa[17, 18] suggesting body fat content to be a major determinant of circulating adiponectin. Lifestyle induced weight loss has been found to increase adiponectin levels among obese women[57], non obese adults[68], insulin resistant obese adults[69] and children[44].

Not all studies have shown an increase in adiponectin with weight loss in obese women[40, 43, 49, 70] obese adults[51], lean and obese women[39, 71] and obese adults with and without MS[41]**.** One study[72] suggested a need for relatively large weight loss for improvement in adiponectin in obese adults**.** Because of the variations due to study population, sample size, differences in baseline weight and the amount of weight lost, the evidence provided by these studies is inconclusive about the effect of weight loss on adiponectin levels.

2.4 ADIPONECTIN AND INSULIN RESISTANCE

Adiponectin levels have been shown to be lower in insulin-resistant individuals[73]. In a study of Pima Indians, those with high adiponectin concentrations were found to be at lower risk of developing type 2 diabetes than those with low concentrations suggesting a potential role of adiponectin in the pathogenesis of type 2 diabetes[9]. A similar inverse relationship between adiponectin levels and incidence of insulin resistance has been shown in follow-up studies among young adults[66], adults[74], adults with impaired glucose-tolerance[75] and elderly men[76]. Interestingly, adiponectin levels were found to be low among individuals with insulin-resistance regardless of their BMI[73] and were inversely associated with insulin resistance independent of obesity among adults[77]. This degree of association between adiponectin and insulin resistance seemed stronger in overweight than in normal-weight adolescents[78] and in adults with the MS than without the MS[79]. Moreover, adiponectin was independently related to all components of the MS in young adults[80]. Similarly, an inverse association between adiponectin levels and the risk for diabetes was reported among a large population of healthy women[81] and a middle-aged population cohort[82].

The findings of these studies suggest that an increase in adiponectin levels may contribute to improved insulin sensitivity and may provide a molecular link between adiposity and diabetes. However, adiponectin levels did not increase despite improved insulin sensitivity in response to moderate weight loss among obese women[73, 83], adults[41, 84] and increase in physical activity among overweight individuals[85] and healthy men[86]. Improvement in insulin sensitivity was not related to adiponectin levels in the intervention and the control groups among adults with impaired glucose tolerance[87]. These inconsistent findings may partly be explained by differences in either the baseline degree of insulin resistance, adiponectin levels and/or the improvement in insulin resistance and adiponectin levels after each intervention. Recent findings have suggested that the improved insulin

sensitivity induced by changes in adiponectin levels may not result from a short term intervention[88]. Hence, there is a substantial need to examine prospectively the association between adiponectin and insulin sensitivity in a population at increased risk of developing diabetes before enrolling in the study.

3.0 WEIGHT LOSS IS MORE IMPORTANT THAN THE TYPE OF DIET IN IMPROVING ADIPONECTIN LEVELS AMONG OVERWEIGHT/OBESE ADULTS

Sushama D. Acharya, MS[1,2] ; Maria M. Brooks, PhD[2]; Rhobert W. Evans, PhD[2];

Faina Linkov, PhD[2,3]; Lora E. Burke, PhD, MPH[1,2]

[1]University of Pittsburgh School of Nursing, Pittsburgh, PA, USA

[2]University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA

[3]University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

(*Manuscript in Preparation*)

3.1 ABSTRACT

Background: Studies have suggested that adiponectin levels may be modified by dietary intake and weight loss.

Objective: To compare the effect of a standard calorie- and fat-restricted diet (STD-D) and a calorie- and fat-restricted lacto-ovo-vegetarian diet (LOV-D) on changes in total and high molecular weight (HMW) adiponectin levels at six months of a behavioral weight loss intervention trial.

Methods: We delivered the 12-month behavioral intervention to both groups; the only difference was that the LOV-D participants were instructed to eliminate meat, poultry, and fish from their diet. Weight, total and HMW adiponectin levels and dietary intake using a 3-Day Food Record were measured.

Results: The sample included 143 adults (STD-D=79; LOV-D=64) and was 88% female, 67% White (mean BMI = 33.7 kg/m^2). We found no differences in weight, total and HMW adiponectin levels between the two groups at baseline. At six months, both groups had significantly increased total (STD-D [+ 7.2 ± 17.8] %; LOV-D [+ 9.4 ± 21.8] %) and HMW adiponectin levels (STD-D [+ 18.5 ± 32.9] %; LOV-D [+ 15.8 ± 34.5] %) (P = < 0.05) with no significant differences between the groups. We found significant associations between weight loss and changes in total (β(s.e) = -0.71(0.27); P = 0.003) and HMW adiponectin (β(s.e) = -1.37(0.47); P = 0.001) levels independent of the diet type. Weight loss at the higher quartile was associated with improvements of total and HMW adiponectin levels (P < 0.05).

Conclusion: Weight loss was associated with improved total and HMW adiponectin levels regardless of the diet type.

3.2 INTRODUCTION

Adiponectin is an adipocytokine with anti-inflammatory properties that may play a role in the development of insulin resistance[6], diabetes[3] and atherosclerosis[11]. At the cellular level, adiponectin has been found to suppress inflammation of the vascular wall[14] and proliferation of vascular smooth muscle cells[15] and also reduce foam cell formation[29]. Additionally, epidemiological studies have suggested that adiponectin also plays an important role in the development and progression of several obesity-related cancers, such as endometrial cancer[89], postmenopausal breast cancer[90] and colon cancer[91]. Adiponectin levels tend to decrease with an increasing body weight and increase with weight loss[3]. There is substantial evidence that the amount and type of foods eaten have profound effects on risk factors for diabetes and atherosclerosis. Hence, it is plausible that adiponectin can also be significantly affected by changes in dietary composition. Thus, it is important to explore if a preventive approach that includes diet is a feasible intervention strategy to improve adiponectin levels.

It has been reported that circulating adiponectin is present in three distinct forms; low molecular weight (LMW), medium molecular weight (MMW) and high molecular weight (HMW) form[24]. Although the precise biological activities of these forms are not well understood, it has been suggested that HMW adiponectin is the most active form of adiponectin[25]. HMW adiponectin has been reported to be an important factor in explaining the metabolic syndrome[26, 27] and is more closely associated with the insulin resistance syndrome than total plasma adiponectin level[25, 26]. Also, HMW adiponectin has been reported to confer a protective effect against coronary artery disease[28]. However, many intervention studies that included a low-calorie diet that induced weight loss have been limited by the measurement of only total adiponectin.

Hence, little is known about the changes in the HMW level relative to the total adiponectin level induced by such interventions.

Epidemiological studies have suggested that increased adiponectin levels are associated with consuming deep yellow vegetables[32], a Mediterranean-type diet[33], having a high score for the dietary pattern characterized by a high intake of fresh fruits[35] and adherence to a healthy dietary pattern as measured by an alternate healthy eating index[36]. A dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products and low consumption of refined cereals was positively associated with adiponectin levels[37]. However, dietary interventions examining the effects of the type of diet on adiponectin have shown conflicting results. Although diet induced weight loss usually improved co-morbidities associated with obesity, the adiponectin levels remained either unchanged[41, 49-52, 56], increased[20, 57, 64, 92] or decreased[47]. Similarly, a few studies of changes in HMW adiponectin levels following an intervention have shown either no changes in distribution of adiponectin forms[93], increase in HMW[24, 28] or increase in all three forms[46]. However, these studies had several limitations, specifically a limited sample size[24, 28, 46, 93] and a brief intervention period[28, 46]. Thus, the evidence provided by these studies is inconclusive about the effects of diet on HMW adiponectin levels.

There is ample evidence that following a vegetarian diet may be beneficial to general and cardiovascular health. This eating pattern has been linked to less weight gain[61], improved lipid profile[62] and increased body leanness[63] compared to those following a carnivorous diet. The literature also suggests that adoption of a vegetarian eating plan may be sustained longer than other weight-reducing eating plans[94]. Recently, Barnard et al.[95] reported similar acceptability between a low-fat vegan diet and a more conventional diabetes-management diet. However, no

identified studies examined the effect of a lacto-ovo-vegetarian diet on adiponectin levels. Therefore, the purpose of this study was to compare the effect of a standard calorie- and fat-restricted diet (STD-D) vs. a lacto-ovo-vegetarian diet (LOV-D) on total and HMW adiponectin levels at 6 months during a 12-month lifestyle intervention intended to achieve weight reduction.

3.3 METHODS

3.3.1 Study Design

This was an ancillary study to the PREFER (Paving the road to everlasting food and exercise routine) trial. The design, recruitment and randomization procedures for the PREFER trial have been described in detail elsewhere [96]. PREFER is an 18-month behavioral weight loss study that examined the effects of 2 dietary options; STD-D vs. LOV-D by whether or not subjects received their preferred dietary treatment (Preference-Yes vs. Preference-No). Participants were randomly assigned first to one of the two preference conditions (yes or no). If assigned to Preference-No condition, they were further randomly assigned to one of the two diet conditions (STD-D or LOV-D). If assigned to Preference-Yes condition, they were assigned to the diet they indicated as preferred at screening.

The present ancillary study merged the data of the two preference groups and compared the changes from baseline to six months between the two diet groups: STD-D and LOV-D. The study protocol was approved by the University of Pittsburgh Institutional Review Board; all participants provided written informed consent and all procedures were followed in accordance with the ethical standard of this board. The PREFER trial was conducted between 2000 and

2005. The current analysis was limited to the 143 participants whose sera samples were available from both baseline and 6-month assessments.

3.3.2 Study Population

Eligibility criteria required that individuals were between 18 and 55 years of age; had a body mass index (BMI) between 27 and 43 kg/m^2 inclusively and had completed a 5-day Food Record at screening. Individuals were excluded if they had a current medical condition requiring physician supervision of diet or physical activity; were pregnant or had intention to become pregnant during the study period; reported alcohol intake of $\geq$ 4 drinks/day; were participating in any weight-loss program in the last 6 months, taking any medication to induce weight loss; or reported abstention from eating meat, poultry, or fish in the past month.

3.3.3 Intervention

The same behavioral intervention was delivered to all treatment groups in the weekly group sessions during the first six months. The only difference was that the LOV-D participants were instructed to eliminate meat, poultry, and fish from their diet by the sixth week of the program. They were permitted to eat dairy products, eggs and meat flavorings. The cognitive-behavioral intervention used several strategies from conceptual models of motivation and behavior change. All participants received nutritional and behavioral counseling to develop skills to implement a healthy lifestyle. Several approaches were used to promote session attendance and adherence to the treatment protocol. Incentives to promote attendance included receiving either *Cooking Light* for the STD-D diet group or *Vegetarian Times* for the LOV-D diet group at the session.

In both groups, the goal of the intervention was to restrict calories and replace total fat intake, especially saturated fatty acids, with increased intake of fruit, vegetables and whole grain products. In addition, participants in the LOV-D were counseled on how to follow a LOV-D and

suggested to incorporate several plant based foods, such as lentils, beans and soy products to replace meat products. Numerous vegetarian foods were sampled in the LOV-D group sessions while the STD-D group sampled low fat foods.

Participants received daily dietary goals based on gender and baseline body weight. For participants weighing < 200 lbs, the prescribed calorie intake was 1200 kcal for women and 1500 kcal for men. For those weighing ≥ 200 lbs, the goal was 1500 kcal for women and 1800 kcal for men. The fat gram goal was 25% of the total calories for everyone. Participants were instructed to increase their physical activity to reach a weekly goal of 150 minutes by week six. We instructed participants to record daily in a paper diary their energy, fat intake and the minutes of physical activity performed. Completed diaries were collected at each session. The interventionists reviewed the diaries and returned them to the participants with written feedback at the next session. The feedback was comprised of suggestions based on personal preference and culture on how the diet could be improved in the respective groups.

3.3.4 Measures

Sociodemographics. Baseline sociodemographic characteristics were collected via a self-administered, standardized questionnaire. Information obtained included age, gender, race, marital status, education and income.

Dietary assessments. At baseline and 6 months, participants were instructed to complete a 3-Day Food Record and asked to record dietary intake on twp work days and one leisure day. Project staff reviewed the diary with the participant to ensure that full detail on foods consumed was provided. The data were analyzed using the Nutrition Data System for Research software (Nutrition Coordination Center, University of Minnesota, Minneapolis, MN) by staff at the

Obesity/Nutrition Research Center who were blinded to the treatment group and assessment period.

Adherence to the LOV-D. Adherence to the LOV-D was based on whether participants reported any meals containing meat, poultry or fish on the 3-Day Food Record at six months (100% adherence: reported no meat, poultry or fish; < 100% adherence: reported ≥ one meal containing meat, poultry or fish).

Physical Activity Assessments. The total energy expenditure for the past seven days was collected via a self-administered Paffenbarger Activity Questionnaire. This questionnaire has been shown to have good test-retest reliability [97]. A metabolic equivalent value was assigned to each leisure activity, from which the total energy expenditure was calculated.

Anthropometric and biochemical measurements. We measured weight on a digital scale **(**Tanita Corporation of America, Inc., IL) with the person in light clothing and without shoes. Height was measured on a wall-mounted standiometer. BMI was computed using the following formula: BMI = weight (kg) /height (meters^2). Blood samples, obtained following a 12-hour overnight fast were assayed at the Heinz Nutrition Laboratory, University of Pittsburgh. Analyses of serum triglyceride and high density lipoprotein (HDL) levels were conducted enzymatically using the ATAC 8000 autoanalyzer (Vital Diagnostics, Lincoln, RI). LDL was estimated using the Friedewald equation. All measures were obtained at baseline and six months.

Adiponectin measurements. The values of total adiponectin and HMW adiponectin were obtained from the stored samples of the PREFER study. Sera from each assessment point were stored at -80^{o}C at the School of Nursing Laboratory. Serum levels of total and HMW adiponectin were determined using an ELISA technique (ALPCO Diagnostics, Salem, NH). The samples (10 µL) were pretreated with separate proteases for determination of total and HMW adiponectin,

respectively. After pretreatment, all samples were incubated for 60 minutes at room temperature (RT) in wells coated with anti-human adiponectin monoclonal antibodies. The plates were washed and a second adiponectin monoclonal antibody, with an independent epitope, and labeled with biotin was added. The plates were again incubated for 60 minutes at RT, washed and horseradish labeled streptavidin added. The plates were incubated for 30 minutes at RT, washed and substrate solution (O-phenyldiamine) added and the plates incubated for 10 minutes at RT. The reaction was stopped with H2SO4 and the absorbance read at 492 nm. Blanks, standards (0.075 to 4.8 ng/mL) and control pools were run with each set of samples. The intra-assay and inter-assay co-efficient of variations (CV) were 6.4% and 12.7% for total adiponectin and 6.4% and 12.6% for HMW adiponectin, respectively. All samples were assayed in duplicate. The analyses were performed at the Heinz Nutrition Laboratory, Graduate School of Public Health at the University of Pittsburgh.

3.3.5 Statistical Analysis

All continuous variables were checked for normality. Data are expressed as mean ± SD or as proportions. Comparisons between the two groups were examined using an independent samples *t*-test, a Mann-Whitney test or a chi-square test. A paired t-test or Wilcoxon's matched pairs test was used for assessing changes within the groups. Associations between variables were assessed with the Pearson or Spearman correlation coefficient. A multiple linear regression was used on percent change in total and HMW adiponectin separately as the dependent variables with the diet types adjusting for age, gender, race, baseline weight, baseline energy expenditure, changes in weight and energy expenditure. Two possible interactions were explored (the diet groups and

weight loss; weight loss and gender) and included in the model only if found to be statistically significant. A one-way analysis of variance was used to compare changes in adiponectin levels by quartiles of weight change. The significance level was set at < .05. Statistical analyses were performed using SAS (version 9.2; SAS Institute Inc, Cary, NC).

3.4 RESULTS

Sera samples of 143 (95%) of the 151 participants who completed the 6-month assessment were available. No differences in baseline characteristics were found among participants who were excluded when compared with participants included in the analysis. The study sample included 143 adults (STD-D = 79; LOV-D = 64) with a mean BMI of 33.7 kg/m^2 and was 88% female, 67% White, and 44.2 ± 8.5 years old. We found no differences in age, education, weight, BMI, dietary intake, total and HMW adiponectin levels between the two groups at baseline (Table 1). Compared to males, females had significantly higher total (7.85 ± 3.32 vs. 6.31 ± 2.58 ug/ml; *P* = .04) and HMW adiponectin levels (3.37 ± 2.9 vs. 2.39 ± 1.43 ug/ml; *P* = 0 .03).

At six months, the STD-D group significantly increased total adiponectin by 7.2 % (*P* = .04) and HMW adiponectin levels by 18.5% (*P* = .001) compared with baseline levels. The LOV-D group also significantly increased total adiponectin by 9.4% (*P* = .01) and HMW adiponectin levels by 15.7% (*P* = .02). However, differences in the 0 – 6 month levels of total adiponectin (*P* = .51) and HMW adiponectin (*P* = .62) between the two groups were not significant (Table 2). Analysis was also conducted excluding participants who reported <100% adherence to the LOV-D (n = 23). There were no statistically significant differences in the

changes of total and HMW adiponectin levels between the STD-D and the 100% adherent LOV-D groups.

There was a significant decrease in body weight within both groups (p for all < .001); with no significant difference in weight loss between the two groups (P = .5). Overall, males lost significantly more weight than females (-13.5 ± 6.5 vs. – 8.1 ± 5.8 %; P < .001) with significantly greater improvement in total (19.8 ± 18.5 vs. 6.7 ± 19.4%; P = .01) and HMW adiponectin levels (41.1 ± 36.9 vs. 14.3 ± 32.0%; P = .002). We observed a highly significant correlation between total and HMW adiponectin levels at baseline (r = .95; P < .0001) and 6 months (r = .95; P < .0001). Total energy intake and fat intake decreased in both groups (P = <.0001) with no differences between the groups. Similarly, both groups increased carbohydrate intake (P = <.0001), but this increase was significantly higher in the LOV-D group (P = .01) than in the STD-D group. The STD-D groups increased protein intake (P = .001) but no change in total protein intake was observed in the LOV-D group. The LOV-D group reduced animal protein intake (P = .004) but the STD-D group increased this intake of this food group (P = .001) (Table 2).

In multiple linear regression analysis, weight loss was significantly and independently associated with changes in total (P = .003) and HMW adiponectin levels (P = .001). However, the diet type was not associated with total (P = .64) or HMW adiponectin levels (P = .48) (Table 3). In order to further examine the effects of weight change on adiponectin, we divided the sample into quartiles of weight change and found a significant increase in total (P = .008) and HMW adiponectin levels (P = .008) with weight change by quartile. Post hoc analyses revealed a significant improvement in total adiponectin level between weight change at the first and the fourth quartile (P < .05) and also between the first and the third quartiles (P < .05). Similarly, a

significant increase in HMW adiponectin level was observed with weight change between the first and the fourth quartile ($P < .01$) (Figure 1).

3.5 DISCUSSION

We found significant increases in total and HMW adiponectin levels in response to both diets, however, the LOV-D did not modify adiponectin levels to a different degree than the STD –D at six months. Weight loss was associated with increased total and HMW adiponectin levels, independent of the diet type. Moreover, we also demonstrated a need for a greater weight loss to significantly improve adiponectin levels in this population.

To our knowledge, this is the first study to compare the effect of STD-D and LOV-D on adiponectin levels. A significant decrease in weight and improvements in adiponectin levels in both groups suggest that both diets are equally effective in weight reduction and improvement of adiponectin levels. Moreover, comparable changes were observed in serum lipids levels in both groups, which may indicate that the type of diet might not be as important and that more than one dietary approach may be followed based on individual preferences and metabolic needs, provided that the weight loss is maintained. However, we cannot rule out the possibility of specific food groups having positive effects on adiponectin levels. In particular, studies have suggested that increased adiponectin levels are observed with consumption of a high intake of dietary fiber[33, 37, 98, 99], deep yellow and green vegetables[32] and moderate alcohol intake[34]. Longitudinal studies are required to determine further relationships between the food groups and adiponectin levels.

Several studies have examined the relationship between dietary interventions and adiponectin levels. No changes were observed in adiponectin level following either a moderate fat/moderate carbohydrate or low fat/high carbohydrate diet in obese women[49]. Similarly, there were no differences in adiponectin levels between the two groups of obese individuals randomly assigned to either a low carbohydrate or a low fat diet[50-52, 100]. Xydakis et al.[41] examined the impact of a VLCD on adiponectin in obese adults with and without the metabolic syndrome and found no change in adiponectin levels in both groups. No differences in adiponectin levels were observed between a low fat diet and the control groups in obese adolescents[56]. In contrast, compared with the control group, a significant increase in adiponectin level was observed following a lifestyle intervention that included a Mediterranean-style diet[57]. A significant increase in adiponectin level was observed after weight loss was induced by gastric bypass surgery[20, 92] and with a calorie-restricted diet among individuals with type 2 diabetes[3]. A high monounsaturated fat diet was associated with greater improvements in adiponectin levels when compared to a high carbohydrate-or protein diet among weight stable hypertensive adults[101].

The possible explanations for these inconsistent findings might be due to variations in type and duration of intervention, sample size, individual variations in adiponectin levels and the populations studied. However, a major consistency found in all studies that failed to show improvements in adiponectin levels was a significant but small weight loss. On the contrary, studies with significant improvements in adiponectin levels had at least a 10% weight loss. These observations have led some investigators to suggest that a threshold of at least a 10% weight loss is essential to observe significant improvements in adiponectin levels[40, 72, 93].

We found a linear relationship with mean weight loss of 8.72% and improvements in total and HMW adiponectin levels; however, a modest clinically meaningful weight loss of 5%

was not enough to observe significant improvements in adiponectin levels. We observed a significant improvement in total adiponectin at the third and fourth quartiles of weight loss while a similar improvement in HMW adiponectin occurred only at the fourth quartile suggesting that weight loss of at least 8% and 12% might be necessary to observe significant improvements in total and HMW adiponectin levels, respectively. Our findings are consistent with the findings of others who have reported a need for substantial weight reduction to increase adiponectin levels[40, 72, 93, 102, 103]. The requirement of a greater weight loss for a marked increase in total and HMW adiponectin levels is further strengthened by our findings that significantly greater improvements in adiponectin levels were paralleled with a significantly greater weight loss among men when compared to women, despite women having significantly higher levels adiponectin levels at baseline. As suggested by Weyer et al.[6], these results indicate a negative feedback mechanism of obesity on adiponectin production, but the size of the adipose tissue might be one of the key determinants of adiponectin levels.

We found more than a two fold increase in HMW adiponectin level when compared with the total adiponectin level (17.3% vs. 8.2%). We also found highly significant associations between total and HMW adiponectin at baseline and six months. Although we did not measure MMW and LMW adiponectin levels separately, it is possible that the increased total adiponectin level might have reflected largely the change in HMW adiponectin. Additionally, a comparable relationship between weight loss and both total and HMW adiponectin levels suggest that a measurement of only total adiponectin might be adequate to examine relationship between the two and that the role of HMW adiponectin level is no more important than that of total adiponectin in relation to weight loss.

The main limitation of this study included the self-reported diet measure. Additionally, dietary data collected with the 3-day Food Records may not represent dietary intake over six months. However, the interventionists reviewed the diaries to provide feedback and confirm that participants complied with the intervention protocol during the rest of the study period. Participants' reporting of meat products in the 3-day Food Records was consistent with what they had reported in the weekly diaries. Hence, we considered the reporting of meat, poultry and fish in the LOV-D group to be reliable indicators of adherence to the LOV-D. Moreover, we did a separate analysis with only those who were 100% adherent to LOV-D group and found no difference, thus it unlikely that bias related to adherence to the LOV-D was present. The small representation of males is another limitation. The strengths of this study included the prospective measurement of both total and HMW adiponectin levels and a comprehensive examination of the effects of diet and weight loss on adiponectin levels with a relatively large sample size for a considerably longer period than reported in the literature. Additionally, data were obtained from a randomized clinical trial.

3.6 CONCLUSION

The study showed the beneficial effects of the intervention on changes in adiponectin levels in both diet groups. These findings suggest that weight loss is associated with improved total and HMW adiponectin levels; however they are independent of the diet type. Moreover, a weight loss closer to 10% might be necessary for significant improvements in adiponectin levels. Thus, focusing on enhancing weight loss might be the better approach to improving adiponectin levels.

Table 3-1 Baseline characteristics by the dietary groups (N = 143)

Variables	STD-D (n = 79)	LOV-D (n = 64)	*P** value
Age (y)	43.5 ± 8.8[2]	45.2 ± 8.1	.22
Female [n (%)]	71 (89%)	56 (88%)	.79
Education (y)	15.4 ± 2.5	15.2 ± 2.4	.67
White [n (%)]	53 (67%)	43 (67%)	.84
Weight (kg)	94.7 ± 15.1	93.7 ± 12.9	.67
BMI (kg/m^2)	33.6 ± 4.1	34.0 ± 3.9	.58
Total cholesterol (mg/dL)	201.8 ± 35.3	203.8 ± 40.4	.76
HDL cholesterol (mg/dL)	54.3 ± 11.7	50.7 ± 10.5	.06
LDL cholesterol (mg/dL)	121.7 ± 33.0	126.1 ± 36.0	.43
Triglycerides (mg/dL)	129.4 ± 74.2	134.8 ± 65.3	.65
Energy intake (kcal)	2011.6 ± 565.9	2079.1 ± 677.1	.52
Total fat (% kcal)	34.8 ± 6.4	34.9 ± 6.9	.90
Carbohydrate (% kcal)	49.4 ± 8.1	50.4 ± 7.2	.42
Protein (% kcal)	16.0 ± 3.6	15.6 ± 3.6	.56
Animal protein (% kcal)	11.2 ± 3.4	10.1 ± 3.5	.15
Energy expenditure (kcal/wk)	2103.6 ± 2054.0	2218 ± 2167	.32
Total adiponectin (ug/ml)	7.8 ± 3.7	7.6 ± 2.8	.72
HMW adiponectin (ug/ml)	3.3 ± 2.2	3.2 ± 1.6	.81

Note, Data are expressed as mean ± SD (except gender).
STD-D = standard weight loss diet; LOV-D = lacto-ovo-vegetarian diet; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; HMW = high molecular weight.
**p*-values are from two sample t-test or Chi-square test.

Table 3-2 Change and percent change score at six months by the dietary groups (N = 143)

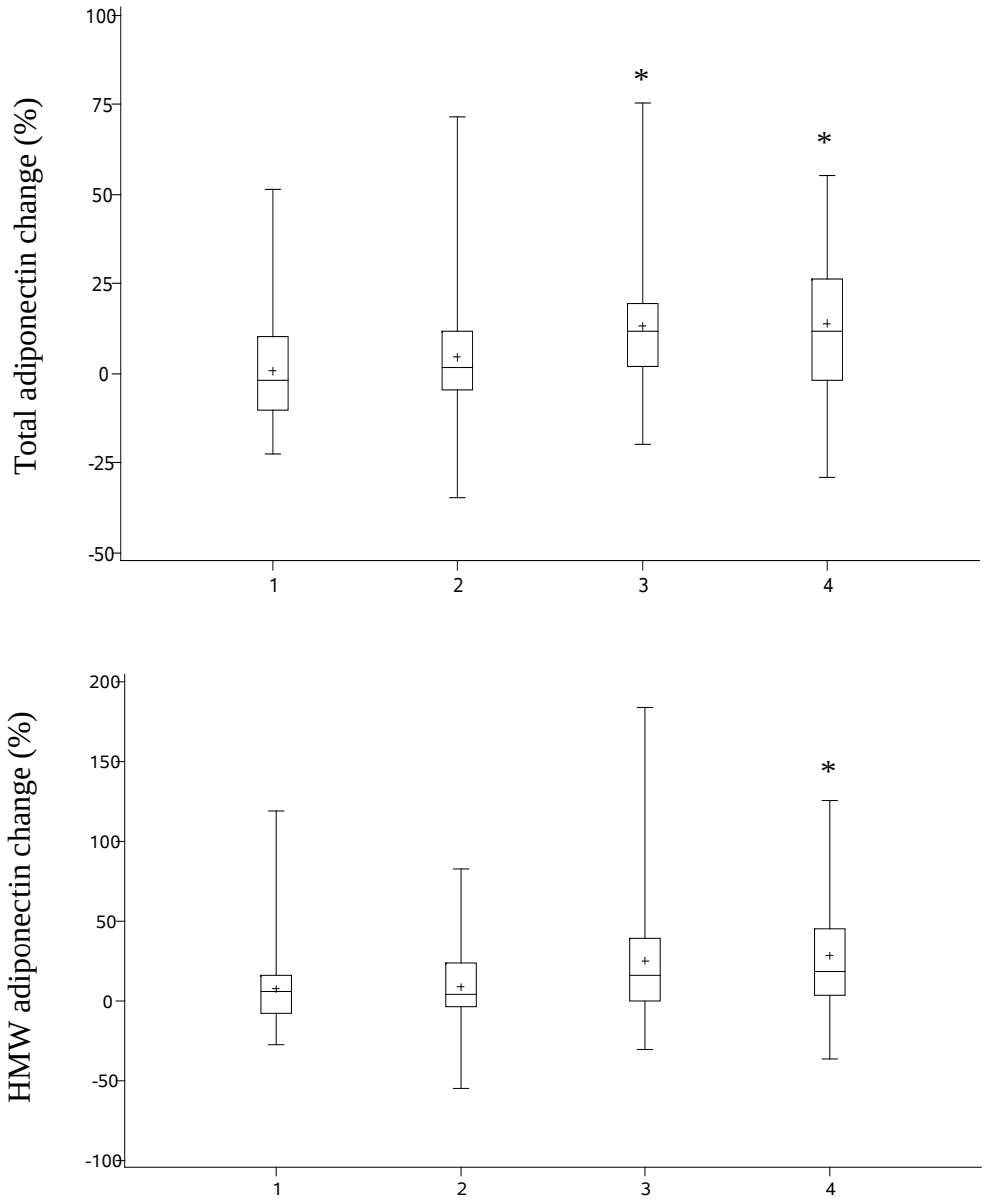
	STD-D (n = 79)		LOV-D (n = 64)		*p** value
	Change	Percent change	Change	Percent change	
Weight (kg)	-8.0 ± 6.6[2]	-8.5 ± 6.3	-8.3 ± 6.0	-9.0 ± 6.0	.80
BMI (kg/m^2)	-2.8 ± 2.1	-8.4 ± 6.3	-3.0 ± 2.0	-9.0 ± 5.9	.51
Total cholesterol (mg/dL)	-3.0 ± 26.6	-.4 ± 13.6	-9.4 ± 28.1	-3.7 ± 12.7	.17
HDL cholesterol (mg/dL)	-2.8 ± 7.5	-4.1 ± 13.3	-2.0 ± 7.9	-2.3 ± 15.6	.56
LDL cholesterol (mg/dL)	3.2 ± 23.0	3.2 ± 18.9	-6.8 ± 22.8	-3.2 ± 15.7	.06
Triglycerides (mg/dL)	-17.3 ± 58.07	-4.7 ± 30.8	-2.6 ± 64.8	-2.8 ± 20.6	.15
Energy intake (kcal)	-602.2 ± 508.1	-26.9 ± 21.5	-674.2 ± 497.5	-29.2 ±17.1	.47
Total fat (% kcal)	-9.4 ± 8.9	-25.3 ±23.5	-11.3 ± 9.6	-29.5 ± 26.5	.31
Carbohydrate (% kcal)	+ 7.3 ± 10.9	+17.6 ± 25.7	+13.0 ± 10.5	+28.2 ± 24.5	.01
Total protein (% kcal)	+2.8 ± 4.6	+22.1 ± 42.7	-0.1 ± 4.5	-3.7 ± 33.7	.0006
Animal protein (% kcal)	+ 1.1 ± 4.8	+18.8 ± 66.1	-3.6 ± 4.7	-28.2 ± 46.1	<.0001
Energy expenditure (cal/wk)	+ 1298 ± 2908	+ 348 ± 874	+ 1249 ± 2812	+ 243 ± 420	.98
Total adiponectin (ug/ml)	+ .4 ± 1.4	+ 7.2 ± 17.8	+ .6 ± 1.8	+ 9.4 ± 21.8	.45
HMW adiponectin (ug/ml)	+ 0.3 ± 0.9	+ 18.5 ± 32.9	+ 0.3 ± 1.0	+ 15.8 ± 34.5	.83

Note, Data are expressed as mean ± SD
STD-D = standard weight loss diet; LOV-D = lacto-ovo-vegetarian diet; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; HMW = high molecular weight
**p*-values are from two sample t-test or or Mann-Whitney test

Table 3-3 Final multiple regression models on changes in adiponectin levels at six months (N = 143)

Measures	Total adiponectin Δ (%)			HMW adiponectin Δ (%)		
	Estimate	s.e.	*p* value	Estimate	s.e.	*p* value
Age (y)	0.64	0.19	.73	0.02	0.32	.94
Gender	-7.96	5.49	.14	-18.03	9.24	.06
Race	0.25	0.26	.3	-0.02	0.15	.8
Diet groups	1.51	3.23	.64	-3.80	5.40	.48
Weight Δ (%)	-0.71	0.27	.01	-1.37	0.47	.004
Baseline weight (kg)	0.02	0.05	.65	0.06	0.09	.50
Energy expenditure Δ (%)	0.0008	0.0006	.20	0.0003	0.001	.76
Baseline energy expenditure (cal/wk)	0.0007	0.0008	.41	-0.0003	0.001	.79

Note, Reference groups = males, White and standard calorie and fat restricted diet
Δ = change; HMW = high molecular weight



Weight change (Range)	+3.5, -4.1	-4.2, -7.9	-8.0, -11.8	-11.9, - 28.3

Figure 3-1 Effect of the level of weight loss on changes in total and HMW adiponectin levels.

HMW, high molecular weight

* Indicates significant differences from the first quartile, $P = < .05$

P-values are from one way ANOVA and tukey post hoc test.

4.0 TOTAL AND HIGH-MOLECULAR-WEIGHT ADIPONECTIN LEVELS IN RELATION TO INSULIN RESISTANCE AMONG OVERWEIGHT/OBESE ADULTS

Sushama D. Acharya, MS[1,2] ; Maria M. Brooks, PhD[2]; Rhobert W. Evans, PhD[2]; Faina Linkov, PhD[2,3]; Lora E. Burke, PhD, MPH[1,2]

[1]University of Pittsburgh School of Nursing, Pittsburgh, PA, USA

[2]University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA

[3]University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

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4.1 ABSTRACT

Background: To determine whether baseline levels or intervention-associated changes in total and high molecular weight (HMW) adiponectin levels were associated with insulin resistance after six months of behavioral treatment for weight loss.

Design: An ancillary study to a behavioral weight loss trial; the intervention was delivered in group sessions.

Methods: Participants included 143 overweight/obese adults with a mean body mass index of 33.7 kg/m^2. The sample was 88% female, 67% White and 44.2 ± 8.5 years old. We measured circulating adiponectin levels (total and HMW) and evaluated the homeostasis model assessment of insulin resistance (HOMA).

Results: At baseline, we found significant inverse associations between total adiponectin and HOMA ($P < 0.001$) and between HMW adiponectin and HOMA ($P < 0.001$) independent of weight. From baseline to six months, there was 17% improvement in HOMA ($P < 0.001$), 8% increase in total adiponectin ($P = 0.001$), 17% increase in HMW adiponectin levels ($P < 0.001$) and 8.72% weight loss ($P < 0.001$). At six months, we found a significant inverse association between changes in total adiponectin and HOMA ($P = 0.04$) that was independent of baseline weight and weight loss. In contrast, the association between changes in HMW adiponectin and HOMA was attenuated after adjustment for weight loss.

Conclusions: An increased level of total adiponectin was associated with improved insulin sensitivity regardless of baseline weight and weight loss. However, baseline total and HMW adiponectin levels were more strongly associated with HOMA than changes in these measures at six months. HMW adiponectin level was not related more closely to insulin resistance than total adiponectin level.

4.2 INTRODUCTION

Adiponectin is one of the many hormones secreted by adipose tissue as the adipokines in the human body. Insulin resistance (IR) and obesity are both associated with lower plasma adiponectin concentrations[17]. Although obesity has long been recognized as a major risk factor for the development of insulin resistance and type 2 diabetes, the mechanism by which adipose tissue contributes to progression of IR and type 2 diabetes is not well understood. Improvements in IR, glucose uptake in skeletal muscles and hepatic fatty acid oxidation upon administration of adiponectin in animal studies suggest that adiponectin may provide a link between adiposity and IR and diabetes[104].

A strong inverse relationship between adiponectin and IR has been reported in population cross-sectional studies[77, 79, 105]. Moreover, longitudinal data also suggest a relationship between lower adiponectin levels and the development of IR and diabetes. In a study of Pima Indians, those with high adiponectin concentration were found to be at a lower risk of developing diabetes than those with a low concentration suggesting a potential role of adiponectin in the pathogenesis of diabetes[9]. Similarly, an inverse association between adiponectin and the risk for diabetes was reported among a large population of healthy women[35], a middle-aged population cohort[82], and adults with insulin-resistance[75]. Mather et al. reported baseline adiponectin as a better marker of diabetes prevention than changes in adiponectin levels over one year in the Diabetes Prevention Program (DPP)[106]. However, no association was found between adiponectin and IR despite improved insulin sensitivity in response to weight loss among obese women[46], obese adults[41] and insulin resistant adults who did not have diabetes[93]. Because of the variations due to population, sample size, differences in baseline degree of insulin resistance and

adiponectin, the evidence provided by these studies on the relationship between IR and adiponectin in the presence of weight loss remains equivocal.

Adiponectin exits in circulation as low molecular weight (LMW), medium molecular weight (MMW) and high molecular weight (HMW) forms[17, 24]. HMW adiponectin has been suggested to be the most active form of adiponectin and more closely associated with IR with an ability to enhance insulin action[25, 26]. However, a major limitation of many epidemiological and intervention studies is the measurement of only total adiponectin level. Thus, little is known about how the relationship between HMW adiponectin and IR might differ from the relationship between total adiponectin and IR. Hence, the aims of this study were to determine whether baseline levels or intervention-associated changes in total and HMW adiponectin levels were associated with IR after six months of a behavioral intervention for weight loss.

4.3 METHODS

4.3.1 Study Design

This was an ancillary study to the PREFER (Paving the road to everlasting food and exercise routine) trial, an 18-month behavioral weight loss study designed to evaluate the effects of treatment preference (Preference-Yes vs. Preference-No) and two dietary treatment options (standard calorie restricted low fat diet (STD-D) vs. lacto-ovo-vegetarian diet (LOV-D). Participants were randomly assigned first to one of the two preference conditions (yes or no). If assigned to the Preference-No condition, they were further randomly assigned to one of the two

diet conditions (STD-D or LOV-D). If assigned to Preference-Yes condition, they were assigned to the diet they indicated as preferred at screening.

Although the PREFER trial randomized participants by treatment preference and diet for a four-group design, the present study was conducted as a single sample without regard to randomized diet or preference condition. The design, recruitment and randomization procedures for the PREFER trial have been described in detail elsewhere[96]. The study protocol was approved by the University of Pittsburgh Institutional Review Board; all participants provided written informed consent. The PREFER trial was conducted between 2000 and 2005, and unthawed aliquots of serum samples were stored at -80^{0}C in the Laboratory at the School of Nursing. The current analysis was limited to the 143 participants whose sera samples were available from baseline and 6-month assessments.

4.3.2 Study Population

The study population included adults between 18 and 55 years of age; had a body mass index (BMI) between 27 and 43 kg/m^2 inclusively and had adequately completed a 5-day food diary at screening. Individuals were excluded if they had diabetes or a medical condition requiring physician supervision of diet or physical activity; were pregnant; participated in a behavioral or pharmacological weight-loss program in the last six months, reported alcohol intake of≥ four drinks/day and reported abstention from eating meat, poultry, or fish in the past month.

4.3.3 Intervention

All four treatment groups received the same standard behavioral intervention; the only difference between the diet groups was that the LOV-D participants were instructed to eliminate meat, poultry and fish form their diet by the sixth week of the program. Details of the intervention have been reported elsewhere[96]. In brief, all participants received a daily energy and fat gram goal based on gender and baseline body weight. For participants weighing less than 200 lbs, the prescribed total energy intake was 1200 kcal for women and 1500 kcal for men. For those weighing more than or equal to 200 lbs, the goal was 1500 kcal for women and 1800 kcal for men. The daily fat gram goal was 25% of the total energy intake. Participants were instructed to increase their physical activity gradually, primarily via walking, until they reached a goal of 150 minutes by week six. The intervention group sessions were held weekly during the first six months. The cognitive-behavioral intervention used several strategies from conceptual models of motivation and behavior change. All participants received nutritional and behavioral counseling and were provided with practical hands-on experience to develop skills to implement a healthy lifestyle. All participants self-monitored their daily energy and fat intake, as well as the physical activity (duration and type) that they performed during the study period.

4.3.4 Measures

Baseline demographic characteristics were collected via a self-administered, standardized questionnaire. All anthropometric, biochemical and adiponectin measurements were obtained at baseline and six months. We measured weight on a digital scale **(**Tanita Corporation of America, Inc., IL) with the individual in light clothing and without shoes. Height was measured with a

wall-mounted stadiometer. Blood samples, obtained following a 12-hour fast were assayed at the Heinz Nutrition Laboratory, University of Pittsburgh. Plasma glucose was measured with the use of the hexokinase-glucose 6-phosphate dehydrogenase enzymatic assay (Sigma Diagnostics, St. Louis, MO) and insulin concentration was measured by a radioimmunoassay kit (Linco Research, St. Charles, MO). Insulin resistance was assessed by the homeostasis model assessment of insulin resistance (HOMA-IR) and was calculated as fasting insulin concentration (U/mL) x fasting glucose concentration (mmol/L)/ 22.5. Serum levels of total and HMW adiponectin were determined using the ELISA technique (ALPCO Diagnostics, Salem, NH). The intra-assay and inter-assay CVs were 6.4% and 12.7% for total adiponectin and 6.4% and 12.6% for HMW adiponectin, respectively. All samples were assayed in duplicate.

4.3.5 Statistical Analysis

Statistical analyses were performed using SAS (version 9.2; SAS Institute Inc, Cary, NC). All continuous variables were checked for normality. Basic statistics were expressed as mean ± SD or as proportions unless otherwise specified. For all continuous variables, comparisons between baseline and 6-month measures were examined using a paired Student's t-test. A chi-square test was performed for comparing the categorical variables at baseline. Associations between continuous variables were assessed using the Pearson or Spearman correlation coefficient. Separate multiple linear regression models were used for HOMA at baseline and change in HOMA score (6-month – baseline scores) as the dependent variables to examine associations between total and HMW adiponectin at baseline and six months. The models were adjusted for age, gender, race, baseline weight, baseline energy expenditure, changes in weight and energy expenditure. All tests performed were two-sided and significance level was set at $P < 0.05$.

4.4 RESULTS

Of the 176 participants randomized at baseline, 151 completed the 6-month assessment and the sera samples of 143 (95%) participants were available from both baseline and 6-month assessments. The majority of participants were female (88%), White (67%), currently married or living with a partner (65%) and employed (94%). The mean age was 44.2 ± 8.5 years old with a BMI range of 26.71 to 42.56 kg/m^2 (Mean= 33.78 kg/m^2) and, on average 15.3 years of formal education. No differences in baseline characteristics were found between participants who were excluded and those included in the analysis.

Table 1 describes the baseline, 6-month and change score measures. At baseline, HOMA did not differ by gender (female vs. male: 4.36 ± 2.23 vs. 4.97 ± 2.24; $P = 0.3$) or by race (white vs. non-white: 4.47 ± 2.34 vs. 4.33 ± 20.00; $P = 0.7$). However, females had significantly higher total (7.85 ± 3.32 vs. 6.31 ± 2.58 ug/ml; $P = 0.04$) and HMW adiponectin levels (3.37 ± 2.9 vs. 2.39 ± 1.43 ug/ml; $P = 0.03$) than males. From baseline to 6 months, there was a 17% reduction in HOMA ($P < 0.001$), an 8% increase in total adiponectin ($P = 0.001$) and 17% increase in HMW adiponectin levels ($P < 0.001$). Further, there was a significant weight loss (8.72%) at six months ($P < 0.001$). We observed highly significant correlations between total and HMW adiponectin levels at baseline (r= 0.95; $P < 0.001$) and also at six months (r= 0.95; $P < 0.001$). At baseline and six months, weight, BMI, glucose, and insulin showed significant positive associations with the HOMA score while total and HMW adiponectin levels showed significant negative association with HOMA (P for all < 0.001).

Separate multiple regression analyses were performed for both baseline and 6-month measures with baseline HOMA and the change in HOMA scores as the dependent variables, respectively. The final model with baseline measures adjusted for weight indicated significant

inverse associations between total adiponectin and the HOMA score ($P < 0.001$) and between HMW adiponectin and HOMA ($P < 0.001$). The estimates for total and HMW adiponectin remained highly significant after adjustment for baseline weight (Table 2). At six months, the final regression model revealed a significant inverse association between changes in total adiponectin and HOMA ($P = 0.04$) that was independent of baseline weight and weight loss. In contrast, higher HMW adiponectin was associated with increased HOMA score without weight loss in the model ($P = 0.02$), but the association was no longer significant after adjustment for weight loss (Table 3). Additional adjustment for energy expenditure at baseline and six months did not alter the results.

4.5 DISCUSSION

A better understanding of the extent of the association of adiponectin with IR in obesity might help clarify mechanisms of insulin sensitivity, which in turn, might be beneficial in identifying preventive approaches to enhance insulin sensitivity and thereby reduce the risk for developing diabetes. Our findings revealed that the higher baseline levels of total and HMW adiponectin levels were significantly associated with lower HOMA score, independent of body weight. At six months, there were significant reductions in the HOMA score and weight, which paralleled the improvements in total and HMW adiponectin levels. The increase in total adiponectin was inversely associated with a decrease in the HOMA score independent of baseline weight and weight change. However, the significant association between changes in HMW adiponectin level and the HOMA score was attenuated after adjustment for weight change.

We observed significant inverse associations of total and HMW adiponectin levels with the HOMA score at baseline even after adjusting for baseline weight indicating an independent association of total and HMW adiponectin levels with the HOMA score regardless of the degree of obesity. The association between total adiponectin and HOMA is consistent with findings from previous studies among individuals with type 2 diabetes[6], a representative community adult population[77] and a community-based cohort with and without the metabolic syndrome[79]. The DPP study also reported an inverse association between baseline total adiponectin levels and future diabetes independent of baseline adiposity among individuals at increased risk for diabetes[106]. Our findings suggest the persistence of an independent role of not only total adiponectin but also HMW adiponectin levels in relation to IR among a generally healthy overweight/obese population with no diabetes or clinical vascular disease.

We also examined the association between changes in total and HMW adiponectin levels and HOMA scores separately after six months of intervention. The degree of unadjusted association between HOMA and total and HMW adiponectin levels were similar at both baseline and six months. We observed intervention-associated significant reductions in weight, the HOMA score and improvements in total and HMW adiponectin levels. The increase in total and HMW adiponectin was associated with the change in weight suggesting that change in total and HMW adiponectin levels might reflect body weight change. The multiple regression analysis showed that an increased level of total adiponectin contributed to improved insulin sensitivity independent of baseline weight and change in weight. Although the estimate for change in total adiponectin decreased by 34% when change in weight was added to the model, these results indicate that improvements in total adiponectin level contributed to improved insulin sensitivity beyond the usual clinical markers of diabetes. The present study highlights the importance of

adiponectin in the development of IR and subsequent development of type 2 diabetes. Thus, interventions, such as the one used in this study, that target weight loss and increase total adiponectin levels may improve insulin sensitivity and reduce the risk of diabetes for overweight individuals.

A few studies have reported HMW adiponectin to have a stronger association than total adiponectin level in the incidence of type 2 diabetes[5, 81] and insulin sensitivity[24-27]. Most of these studies either had only a single measurement of HMW adiponectin level[5, 26, 27, 81],were limited by the small sample size[24, 25, 93], or had a pharmacological intervention[107]. We found a highly significant association between total and HMW at baseline. Bluher et al.[108] reported no superiority of HMW adiponectin over total adiponectin values in predicting insulin sensitivity. Likewise, we also showed a similar magnitude of correlations between both total and HMW adiponectin levels with the HOMA score at baseline suggesting no clinical differences between total and HMW adiponectin levels in relation to insulin resistance.

At six months, the significant association between changes in HMW adiponectin level and HOMA was attenuated after adjustment for change in weight and was no longer significant despite an increase in HMW adiponectin that was more than double the increase in total adiponectin. This finding indicates a possible threshold level of HMW adiponectin that might be needed to have an independent effect on change in the HOMA score. Alternatively, we cannot rule out the possibility of a different pathway of an intervention-associated change in HMW adiponectin level from the baseline HMW adiponectin level. Regardless of the mechanism, our study refutes the hypothesis of a predominant role of HMW adiponectin level over total adiponectin level in relation to IR. The study also provided us a unique opportunity to examine if baseline or intervention associated changes in total and HMW adiponectin levels were more

strongly associated with IR. We found that the degree of association between total and HMW adiponectin level were stronger at baseline than the relationship between changes in total adiponectin and the HOMA score at six months. Moreover, the significant association between change in HMW adiponectin level and HOMA score was taken over by weight loss. Consistent with the findings of Mather et al.[106] we also found that the baseline total and HMW adiponectin levels were more closely related to IR than the changes in these measures at six months.

The potential mechanism of how plasma adiponectin levels influence IR is still not known. A higher level of adiponectin level has been reported to increase fatty acid oxidation with subsequent reduction of triglycerides thereby directly sensitizing the body to insulin and reserving?? IR in animal models of obesity and diabetes[109] and in humans[27]. Through in vitro studies, adiponectin has been shown to trigger the protein kinase, an insulin independent enzyme known to stimulate glucose use and increase fatty acid oxidation in skeletal muscle[104]. It has been reported that some inflammatory markers, such as tumor necrosis factor-α, interleukin-6 or C-reactive protein have an adiponectin inhibitory effect, which in turn may lead to IR[105, 110]. It is possible that the levels of these markers decrease with the corresponding increase in adiponectin levels with weight loss and may improve insulin sensitivity.

A main limitation of the study included a measure of IR with a surrogate marker. However, HOMA derived from the mathematical model has been shown to be an adequate indicator of IR and has been used in many epidemiological and clinical studies. Also, the generalizability of these findings may be limited due to the relatively homogenous study population; however, the minority representation exceeded that of the local community. The strengths of the study included the measurements of both total and HMW adiponectin. An additional strength was its longitudinal design and examination of the relationship between total

and HMW adiponectin levels with IR cross-sectionally and prospectively. Moreover, the clinical measures were obtained via a standardized protocol and assays were performed with good precision and the study included a larger sample than what has been reported in the literature.

4.6 CONCLUSION

An increased level of total adiponectin contributed to improved insulin sensitivity regardless of baseline weight and weight loss. However, the association of change in total adiponectin and HOMA score was modest compared to baseline measures suggesting that the cross-sectional measure of total adiponectin might be a comparable indicator of IR and subsequent risk of developing diabetes among overweight or obese adults. These findings provide support for the importance of adiponectin levels and a need for their improvements to prevent diabetes. Thus, interventions that enhance adiponectin secretion or action may have potential for diabetes risk reduction. Improvements in the HMW adiponectin level were largely explained by the weight loss suggesting the role of HMW adiponectin level is no more important than that of total adiponectin in relation to improved insulin sensitivity.

Table 4-1 Baseline, six months and percent change scores in measures (N = 143)

	Baseline means ± SD	6-mo means ± SD	6-mo % change means ± SD	P^1
Weight (kg)	94.26 ± 14.10	86.10 ± 14.45	-8.16 ± 60.05	< 0.0001
BMI (kg/m^2)	33.78 ± 40.04	30.88 ± 4.54	-2.91 ± 20.01	< 0.0001
Glucose (mg/dl)	96.13 ± 8.32	95.41 ± 8.35	-0.73 ± 8.67	0.31
Insulin (uU/mg)	18.42 ± 8.35	14.23 ± 5.96	-4.18 ± 5.88	< 0.0001
HOMA	4.43 ± 2.23	3.40 ± 1.58	-10.03 ± 1.64	< 0.0001
Adiponectin (ug/ml)	7.68 ± 3.27	8.11 ± 3.13	0.44 ± 1.58	0.0013
HMW (ug/ml)	3.26 ± 1.96	3.58 ± 1.90	0.32 ± 0.93	< 0.0001

Note, BMI, body mass index; HOMA, homoeostasis model assessment of insulin resistance; HMW, high molecular weight.
[1]significantly different at six months from baseline measures.

Table 4-2 Multiple regression models of total and HMW adiponectin levels on baseline HOMA measure (N = 143)

Models	Baseline HOMA			R^2	Models	Baseline HOMA			R^2
	Estimate	s.e.	*P*			Estimate	s.e.	*P*	
Model 1					**Model 1**				
Adiponectin (ug/ml)	-0.28	0.05	< 0.001	0.17	HMW (ug/ml)	-0.42	0.09	< 0.001	0.14
Model 2					**Model 2**				
Adiponectin (ug/ml)	-0.29	0.05	< 0.001	0.18	HMW (ug/ml)	-0.45	0.09	< 0.001	0.15
Gender	-0.12	0.55	0.83		Gender	-0.10	0.56	0.85	
Race	-0.40	0.37	0.28		Race	-0.52	0.38	0.17	
Age (y)	-0.02	0.02	0.27		Age (yr)	0.01	0.02	0.40	
Model 3					**Model 3**				
Adiponectin (ug/ml)	-0.26	0.05	< 0.001	0.30	HMW (ug/ml)	-0.38	0.09	< 0.001	0.27
Gender	0.45	0.34	0.39		Gender	0.44	0.54	0.42	
Race	-0.46	0.01	0.18		Race	0.55	0.36	0.12	
Age (y)	0.02	0.02	0.20		Age (yr)	0.02	0.02	0.32	
Weight (kg)	0.06	0.01	< 0.001		Weight (kg)	0.06	0.01	< 0.001	

Note, HMW, high molecular weight; HOMA, homoeostasis model assessment of insulin resistance.
Reference groups: gender-males, race- White

Table 4-3 Multiple regression models of total and HMW adiponectin levels on change in HOMA at six months (N= 143)

Models	Change in HOMA				Models	Change in HOMA			
	Estimate	s.e.	*P*	R^2		Estimate	s.e.	*P*	R^2
Model 1					**Model 1**				
Adiponectin Δ (ug/ml)	-0.25	0.09	0.004	.06	HMW Δ (ug/ml)	-0.34	0.14	0.02	0.04
Model 2					**Model 2**				
Adiponectin Δ (ug/ml)	-0.25	0.08	0.005	0.11	HMW Δ (ug/ml)	-0.32	0.15	0.03	0.09
Gender	0.95	0.43	0.03		Gender	0.99	0.43	0.02	
Race	0.38	0.29	0.20		Race	0.33	0.30	0.28	
Age (y)	0.01	0.01	0.9		Age (y)	0.0003	0.01	0.98	
Model 3					**Model 3**				
Adiponectin Δ (ug/ml)	-0.26	0.09	0.003	0.16	HMW Δ (ug/ml)	-0.34	0.15	0.02	0.14
Gender	0.64	0.43	0.14		Gender	0.68	0.44	0.12	
Race	0.44	0.29	0.13		Race	0.38	0.29	0.19	
Age (y)	-0.000	0.02	0.97		Age (y)	-0.001	0.02	0.92	
Baseline weight (kg)	-0.03	0.009	0.003		Baseline weight (kg)	-0.03	0.009	0.003	

Table 4-4 Contd.

Models	Change in HOMA			R^2	Models	Change in HOMA			R^2
	Estimate	s.e.	*P*			Estimate	s.e.	*P*	
Model 4					**Model 4**				
Adiponectin Δ (ug/ml)	-0.17	0.08	0.04	0.27	HMW Δ (ug/ml)	-0.18	0.14	0.21	0.26
Gender	0.06	0.42	0.88		Gender	0.10	0.48	0.82	
Race	0.46	0.27	0.09		Race	0.40	0.27	0.14	
Age (y)	0.0005	0.01	0.97		Age (y)	0.000	0.01	0.99	
Baseline weight (kg)	-0.03	0.009	0.005		Baseline weight (kg)	-0.02	0.008	0.006	
Weight Δ (kg)	0.10	0.02	< 0.0001		Weight Δ (kg)	0.10	0.02	< 0.0001	

Note, HMW, high molecular weight; HOMA, homoeostasis model assessment of insulin resistance; Δ, change.
Changes values were defined as six months – baseline.
Reference groups: gender-males, race- White

5.0 RELATIONSHIPS BETWEEN WEIGHT, WAIST CIRCUMFERENCE, BODY COMPOSITION AND ADIPONECTIN LEVELS: A LONGITUDINAL ANALYSIS AMONG OBESE/OVERWEIGHT ADULTS

Sushama D. Acharya, MS[1,2] ; Maria M. Brooks, PhD[2]; Rhobert W. Evans, PhD[2];

Faina Linkov, PhD[2,3]; Lora E. Burke, PhD, MPH[1,2]

[1]University of Pittsburgh School of Nursing, Pittsburgh, PA, USA

[2]University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA

[3]University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

(Manuscript in Progress)

5.1 ABSTRACT

Objectives: The study assessed the longitudinal relationships between weight, waist circumference, body composition and adiponectin levels at six and 12 months of a behavioral intervention for weight loss.

Methods and Results: The sample included 133 overweight/obese adults with a mean BMI of 33.4 ± 4.7 kg/m^2 and was 86% female, 81% White, and was on average, 45.3 ± 8.8 years old. We measured circulating adiponectin level, weight, waist circumference and body composition at baseline, six and 12 months. Retention at six months was 91% and 85% at 12 months. Adiponectin level increased by 23.4% and 20.1% relative to the baseline value at six and 12 months, respectively (P<0.0001). There were significant reductions in weight (-6.79 ± 6.39%), waist circumference (-6.33 ± 5.93%) and percent body fat (-5.29 ± 9.70%) at six months (Ps for all <0.0001) with no significant differences between six and 12 months. Significant effects of weight loss and reductions in BMI, waist circumference and body fat were found on increased adiponectin levels over time (Ps for all <0.002) However, no associations were observed between changes in fat free mass with adiponectin levels over time.

Conclusions: Reductions in body weight, BMI, waist circumference, and percent body were strongly associated with increases in adiponectin levels over time. Interventions that target a long term weight loss help enhance adiponectin levels.

5.2 INTRODUCTION

Obesity is a disorder characterized by chronic mild inflammation in addition to its association with cardiovascular disease, insulin sensitivity and diabetes. Adiponectin is one of the many adipokines secreted by the adipose tissue that may act as an anti inflammatory protein with some protection against diabetes and atherosclerosis. A number of studies have reported decreased adiponectin level in obese individuals with higher body mass index (BMI) when compared to normal weight individuals[7, 8, 111]. Several intervention studies have described significant improvement in adiponectin level with diet or lifestyle induced weight loss[57, 64, 92] while others failed to find such improvement in spite of significant weight loss[41, 49-52, 56].

A strong inverse relationship of visceral adipose tissue (VAT) and body fat mass with adiponectin has been reported in population studies[111,112]. Similarly, an inverse association between waist circumference and adiponectin has also been reported [19, 111]. However, a major limitation of these studies was the cross-sectional design. Recently, a study by Okauchi et al.[113] found improvement in adiponectin level with reductions in BMI, waist circumference and visceral fat accumulation among middle aged individuals in the general population. No previous studies have reported longitudinal relationships between weight loss and adiponectin levels using repeated assessments among overweight/obese individuals. Hence, the aims of this study were to assess the longitudinal relationships of weight, waist circumference and body composition with adiponectin levels at six and 12 months of participation in a randomized clinical trial that included a behavioral intervention for weight loss.

5.3 METHODS

5.3.1 Study Design and Population

This was a secondary analysis of the 6- and 12-month data from the Self-Monitoring and Recording with Technology (SMART) weight loss trial. SMART was a 24-month randomized clinical trial designed to determine the effects of different methods of self-monitoring on weight loss in which participants were randomized to using one of the three self-monitoring methods: paper diary, or personal digital assistant (PDA), or PDA with the same software plus a tailored feedback program. The study protocol was approved by the University of Pittsburgh Institutional Review Board. All participants provided written informed consent.

The design, recruitment and randomization procedures for the SMART trial have been described in detail elsewhere[114]. In brief, the study recruited three cohorts of individuals from the community between 2006 and 2008 for a total of 210 participants. Individuals were eligible to participate if they were between 18 and 59 years old with a BMI between 27 and 43 kg/m^2 and were able to record in a 5-day food diary at screening. Study exclusion criteria included pregnancy, individuals with conditions that required medical supervision of diet or exercise and those who participated in a weight-loss program in the six months prior to recruitment or planned an extended vacation or relocation during the study period. The current analysis was limited to 133 participants from the first and second cohorts of the SMART trial whose sera samples were available from baseline assessment and the analysis was conducted as a single sample without regard to randomized treatment condition.

5.3.2 Intervention

All three treatment groups received the same standard behavioral intervention; the only difference was in the self-monitoring method they were assigned to use. Details of the intervention have been reported elsewhere[114]. The intervention during the 12 months was delivered in 16 weekly and 16 bi-weekly group sessions. The sessions were held separately for the three treatment groups and the same multidisciplinary team (behavioral scientist, nutritionist and exercise specialist) led the sessions. All participants received nutritional and behavioral counseling and were provided with practical hands-on experience to develop skills to implement a healthy lifestyle. All participants received a daily calorie and fat gram goal based on gender and baseline body weight. For participants weighing less than 200 lbs, the prescribed daily total calorie intake was 1200 kcal for women and 1500 kcal for men. For those weighing more than or equal to 200 lbs, the goal was 1500 kcal for women and 1800 kcal for men. The daily fat gram goal was 25% of the total daily calories for everyone. Participants were instructed to reach a weekly goal of 150 minutes of moderate intensity exercise by sixth week and 180 minutes by the 24^{th} week (six months). They were also instructed to add three 30-minute resistance exercise sessions per week at six months. All participants self-monitored their daily energy and fat intake, as well as physical activity (duration and type) during the study period.

5.3.3 Measures

Demographics. Baseline demographic characteristics were collected via a self-administered, standardized questionnaire. Information obtained included age, gender, race, marital status, education and income.

Anthropometric measurements. All measures were obtained at baseline and repeated at six and 12 months after an overnight fast. A Tanita scale and body fat analyzer **(**Tanita Corporation of America, Inc., Illinois, USA) wa**s** used for weight and body composition. Body composition was assessed via bioelectrical impedance analysis (BIA). Foot to foot BIA was conducted using the Tanita and participants were measured standing erect with bare feet on the analyzer footpads. Height was measured on a wall-mounted standiometer. BMI was computed using the following formula: BMI = weight (kg) /height (meters^2). Waist circumference was measured at least twice with a Gulick II measuring tape. The tape was placed around the bare abdomen just above the hip bone and the measurement was taken after breathing out normally. When two measurements were obtained that were within two cm of each other, the average of the two measurements was used.

Adiponectin measures. Blood samples were obtained following a 12-hour fast. Sera from each assessment point were stored at -80^{o}C at the laboratory at School of Nursing. Serum level of total adiponectin was determined using the ELISA kit (Millipore, Billerica, MA). All samples were assayed in duplicate. The analyses were performed at the R. Tracy Laboratory, University of Vermont.

5.3.4 Statistical Analysis

All continuous variables were checked for normality. Basic statistics were expressed as mean ± SD for continuous variables and as proportions for categorical variables unless otherwise specified. Differences in baseline adiponectin value by gender, race, smoking status and medication use were examined with a two sample Student t-test. For all the studied variables, the changes from baseline to each time point were expressed as scores (Δ = 6 or 12 months -

baseline) and the changes between six and 12 months were expressed as scores (Δ = 12 months - 6 months). To investigate relationships of changes in weight, BMI, waist circumference and body composition (percent body fat, fat free mass) with changes in adiponectin level, we applied repeated-measures analysis using linear mixed effects modeling via PROC MIXED in SAS. The unadjusted models included the main effects for changes in weight, BMI, waist circumference, body composition, time and their interaction. Five possible interactions were tested (Δ weight and time; Δ BMI and time; Δ waist circumference and time; Δ percent fat and time; Δ FFM and time) and included in the final model only if found to be statistically significant. Separate models were conducted for each physical measure. The adjusted models included age, gender, ethnicity, baseline adiponectin level and each baseline physical measure. Additional adjustment included smoking status.

Although 133 sera samples were available from baseline assessment, mixed model analyses were performed on 122 participants who had at least one measure of adiponectin level at either the six or 12 months assessment. Data from 122 participants were used regardless of their participation in all three assessments. Models were based on 206 observations across the assessments. Physical measurements and adiponectin values were missing for some participants; the missing data were mainly due to participant attrition and unavailability of stored sera samples that happened at different time points during the study. Thus, it was assumed that these data were missing at random. Based on the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), unstructured covariance structures were used to fit the model. The restricted maximum likelihood algorithm was used in all the models. Sensitivity analyses were conducted for outliers identified through graphical methods. When outliers were omitted via sensitivity analysis, the results did not change, supporting the robustness of our findings. All tests

performed were two-sided and significance level was set at $P < 0.05$. Statistical analyses were performed using SAS (version 9.2; SAS Institute Inc, Cary, NC).

5.4 RESULTS

Overall, sera samples of 133 (98%) of the 136 participants who completed the baseline assessment were available. For this secondary analysis, the retention was at 91% followed by 85% at 6 and 12 months. The sample was 86% female, 81% White, and 45.29 ± 9.76 years old with BMI of 34.68 ± 4.65kg/m^2 and on average completed 15.53 ± 3.01 years of formal education. Thirty participants (23%) were overweight and 103 were obese (77%) according to the criteria set by the World Health Organization. The proportion of current smokers was 10% (n=13). The number of participants taking medication for high cholesterol was 17 (13%) at baseline and remained 15 (11%) at 6 and 12 months. We found no differences in baseline adiponectin level by gender (male: 14.36 ± 8.88 vs. female: 13.90 ± 6.95 ug/ml; $P = 0.81$), race (White: 14.20 ± 7.36 vs. nonwhite: 13.12 ± 6.58 ug/ml; $P = 0.50$), smoking status (current smokers: 11.62 ± 5.30 vs. non smokers 14.26 ± 7.35 ug/ml; $P = 0.21$) and medication use for high cholesterol (medication users: 13.90 ± 6.95 vs. medication non-users 14.95 ± 7.82 ug/ml; $P = 0.5$).

Table 1 shows the descriptive values of all studied variables at each time point (baseline, six and 12 months). Increase in mean adiponectin level was 23.43% and 20.11% at six months and 12 months, respectively, relative to the baseline value ($P <0.0001$) with no significant difference between changes at six and 12 months. There were significant reductions in weight (-6.79 ± 6.39%), BMI (-6.59 ± 6.39%), waist circumference (-6.33 ± 5.93%), percent body fat (-

5.29 ± 9.70%) and fat free mass (FFM) (- 2.94 ± 5.42%) at 6 months (*P*s for all <0.001). Changes in weight and reduction in BMI, waist circumference and body composition were not significant between six and 12 months.

In the longitudinal analysis with adiponectin at six and 12 months as the dependent variable, we found no significant interactions between time and changes in weight (F = 0.16; *P* = 0.69), BMI (F = 0.19; *P* = 0.66), waist circumference (F = 0.02; *P* = 0.88), percent body fat (F = 0.22; *P* = 0.63), FFM (F = 0.46; *P* = 0.50). The model with changes in weight adjusted for time, age, gender and race indicated a significant increase in adiponectin levels over 12 months with weight loss (β(s e) = -0.15(0.05), F = 9.30; *P* = 0.002) (Tables 2 and 7). When the model was replaced with changes in BMI substituting for weight, the association between changes in BMI and adiponectin levels over time remained significant (β(se) = -0.39(0.13), F = 8.67; *P* = 0.003) (Tables 3 and 7).

Significant associations were observed between reduction in waist circumference (β(se) = -0.13(0.05), F = 7.68; *P* = 0.006) (Tables 4 and 7) and percent body fat (β(se) = -0.21 (0.07), F = 9.29; *P* = 0.002) (Tables 5 and 7) and increased adiponectin levels over time in the separate models adjusted for age, race and gender. However, no such significant association was found between changes in FFM and adiponectin levels over time (β(se) = -0.01(0.04), F = 0.00; *P* = 0.76) (Tables 6 and 7). Additional adjustment for baseline smoking status in all the models with each physical measure did not alter the results (Tables 2-7).

5.5 DISCUSSION

Participants in the 12-month behavioral intervention for weight loss experienced significant reductions in weight, waist circumference and body fat, which were paralleled with increased adiponectin levels. We observed a significant increase in adiponectin levels over time with reductions in weight, BMI, waist circumference, and body fat; no such association was found between changes in FFM and adiponectin levels suggesting adipose tissue as the source of adiponectin levels and a negative feedback mechanism of obesity on adiponectin production.

The maximum reduction in weight, BMI, and waist circumference and greatest improvement in adiponectin level occurred during the first six months followed by maintenance of these measures during the second six months; similar patterns of changes in weight and BMI have been reported in previous studies [115, 116]. Despite recommending participants to add resistance exercise to attenuate fat-free tissue loss and increase strength and function, we observed significant reduction in FFM at six months, which was maintained at 12 months. Dixon et al. [117] reported the expected ratio of fat-free mass loss to fat mass loss is 1.34 during intentional weight loss among adults. In our study this ratio was 1.41; more favorable than the expected ratio. As suggested by Heymsfield et al., [118] this study adds to the evidence that an intentional weight loss also reflects the loss of body protein in addition to body fat loss. However, the loss of body protein may diminish in magnitude over time.

Our study demonstrated a significant association between increased adiponectin levels and a reduction in body fat during a 12-month intervention for weight loss. In contrast, changes in FFM showed no association with changes in adiponectin. Our findings of a significant negative association between adiponectin and body fat have been reported in cross-sectional studies [6, 111, 119, 120]. The non-significant findings between FFM and adiponectin found in the

study strongly suggest that the secretion of adiponectin is not regulated by FFM but rather by the negative feedback of obesity on adiponectin production. As suggested by Weyer et al.[6] and Haval et al.[121], the size of the adipose tissue might also be one of the key determinants of adiponectin level. These cumulative results provide further evidence that the production and secretion of adiponectin is modified by the amount and size of adipose tissue.

We also examined the relationships between changes in waist circumference, weight and BMI with adiponectin level and found that a reduction in waist circumference, weight and BMI was associated with improvement in adiponectin level over time. Several cross-sectional studies have reported a negative association between waist circumference and adiponectin levels [66, 111, 122]. We confirmed these findings in this longitudinal study using repeated measures. Although our measure of body composition with BIA did not differentiate between visceral and subcutaneous adipocytes, it has been known that central fat, as measured by waist circumference is considered a surrogate marker for visceral adipocytes [123]. In a cross-sectional study, Nakamura et al.[124] reported an inverse association between visceral adipocytes and adiponectin. Recently, evidence indicating abdominal adiposity is more strongly associated with diabetes and heart disease than overall adiposity measured by BMI or weight is accumulating [125-127]. Kanaya et al.[128] reported increased risk of diabetes with increasing level of visceral fat among the Health ABC study participants. Similarly, adverse metabolic risk profiles have also been reported with an increasing proportion of visceral adipose tissue [129, 130]. Hence, associations between visceral adipose tissue and diabetes or cardiovascular diseases reported in previous studies may be partly explained by the negative association between changes in waist circumference and adiponectin level. Our findings from this longitudinal study provide data to suggest that adiponectin might be one of the many links between obesity, diabetes and CVD.

A potential biological mechanism of how adiponectin influences diabetes and heart disease is still not known. The findings of our study suggest reduced adiponectin level with increasing adiposity, in particular body fat and central adiposity. It has been hypothesized that the increased release of pro-inflammatory cytokines from adipose tissue with reduced secretion of anti-inflammatory adipokines, such as adiponectin, can generate a low grade chronic inflammatory state. This imbalance of pro- and anti- inflammatory markers might play a role in the future development of diabetes and cardiovascular diseases through reduced insulin sensitivity and endothelial dysfunction. Lifestyle factors such as over eating and physical inactivity induce fat accumulation, which results in dysfunction of adipose tissues. Hence, current findings also emphasize the importance of focusing on long term weight loss accompanied by reduction of total body fat loss and central obesity as an essential preventive measure for optimal health.

We used BIA using Tanita to evaluate body composition in the present study, which is not considered the gold standard. However, significant correlations between measurements obtained from Tanita and dual-energy x-ray absorptiometry and under water weighing have been reported[131]. Recently, Sai Krupa Da[132] reported accuracy and reliability of measuring body composition with BIA and suggested its application as a screening tool in clinical practice and in population based research. Moreover, we standardized measurement conditions to an overnight fast and normal levels of hydration for each measurement, thus we feel confident that the prediction error of the BIA method was at $\leq 4\%$[133]. The relatively homogenous study population might have limited the generalizability of our findings; however, 22% minority in the study sample represents the regional population. Another important limitation was a 15% male representation. The study population presented in this study represents the gender composition of

individuals seeking weight loss treatment as the proportion of males is similar to what has been reported by other weight loss studies[134-136].

The main strength of the study was that it was the first study to include the repeated measures of both adiponectin level and physical measures and examine their relationships over time. Moreover, the clinical measures were obtained via a standardized protocol and assays were performed with good precision and the study included a larger sample than what has been reported in the intervention studies. An additional strength was our 91% retention rate at 6 months and 85% retention at 12 months, which was comparable or better than rates reported for other weight-loss studies.

5.6 CONCLUSION

In conclusion, changes in body composition continuously affected the level of adiponectin over time. We observed beneficial effects of the behavioral intervention on improvement in adiponectin level with reductions in weight, waist circumference and percent body fat. Reductions in weight, BMI, waist circumference, and body fat increased adiponectin levels. As adipose tissue might be the key determinant in the secretion of adiponectin, interventions such as behavioral weight loss interventions accompanied by total fat loss and reduced central obesity may enhance serum adiponectin levels.

Table 5-1 Physical measures and adiponectin level at baseline, six and 12 months

Measures	Baseline (N=133)	6 months (n=121)	12 months (n=113)
	Mean ± SD (min, max)	Mean ± SD (min, max)	Mean ± SD (min, max)
Weight (kg)	94.38 ± 15.87 (66.36, 143.27)	88.61 ± 16.28 (56.18,138.45)	88.28 ± 17.17 (54.64, 135.82)
BMI (Kg/m^2)	34.35 ± 4.69 (26.53, 44.53)	32.22 ± 5.01 (21.69, 43.84)	32.10 ± 5.27 (221.09, 44.12)
WC (cm)	106.17 ± 13.01 (78.75, 138.75)	99.83 ± 13.79 (68.50, 138.50)	100.10 ± 13.69 (67.00, 138.25)
Body fat (%)	42.66 ± 5.86 (20.40, 52.60)	40.37 ± 7.45 (19.30, 54.00)	39.84 ± 7.84 (18.20, 63.10)
Fat free mss (kg)	53.35 ± 9.22 (39.91, 84.23)	51.78 ± 8.72 (39.82, 80.18)	51.75 ± 9.2 (40.27, 79.28)
Adiponectin (ug/ml)	14.00 ± 7.20 (2.42, 48.05)	16.21 ± 7.42(4.24, 44.85)	16.47 ± 7.81 (4.45, 46.54)

Note, BMI, body mass index; WC, waist circumference

Table 5-2 Relationships between changes in weight and adiponectin: mixed models

		F vaule	*P* value
Model 1	Weight Δ (kg)	2.46	0.11
	Time	1.15	0.31
	Weight Δ *time	0.51	0.60
Model 2	Weight Δ (kg)	9.30	0.002
	Baseline weight (kg)	0.01	0.90
	Baselineadiponectin (ug/ml)	516.28	<0.0001
	Time	0.01	0.93
	Age	3.11	0.08
	Gender	1.78	0.18
	Race	0.02	0.87
Model 3	Weight Δ (kg)	9.08	0.003
	Time	0.01	0.91
	Baseline weight (kg)	0.07	0.79
	Baseline adiponectin (ug/ml)	511.82	<0.0001
	Age	2.72	0.10
	Gender	1.85	0.17
	Race	0.04	0.84
	Smoking status	0.61	0.43

Note, Reference groups: Time (baseline); Gender (female); Race (White); Smoking status (smokers)

Table 5-3 Relationships between changes in BMI and adiponectin: mixed models

		F vaule	P value
Model 1	BMI Δ (kg/m2)	2.50	0.11
	Time	2.49	0.08
	Weight Δ *time	1.31	0.27
Model 2	BMI Δ (kg/m2)	8.67	0.003
	Baseline BMI (kg/m2)	0.14	0.70
	Baseline adiponectin (ug/ml)	512.63	<0.0001
	Time	0.00	0.96
	Age	2.92	0.09
	Gender	2.95	0.08
	Race	0.01	0.90
Model 3	BMI Δ (kg/m2)	8.58	0.004
	Baselin BMI (kg/m2)	0.22	0.64
	Baseline adiponectin (ug/ml)	509.14	<0.0001
	Time	0.01	0.94
	Age	2.54	0.11
	Gender	2.83	0.09
	Race	0.03	0.86
	Smoking status	0.72	0.39

Note, BMI, body mass index
Reference groups: Time (baseline); Gender (female); Race (White); Smoking status (smokers)

Table 5-4 Relationships between changes in waist circumference and adiponectin: mixed models

		F vaule	*P* value
Model 1	BMI Δ (kg/m^2)	2.50	0.11
	Time	2.49	0.08
	Weight Δ *time	1.31	0.27
Model 2	BMI Δ (kg/m^2)	8.67	0.003
	Baseline BMI (kg/m^2)	0.14	0.70
	Baseline adiponectin (ug/ml)	512.63	<0.0001
	Time	0.00	0.96
	Age	2.92	0.09
	Gender	2.95	0.08
	Race	0.01	0.90
Model 3	BMI Δ (kg/m^2)	8.58	0.004
	Baselin BMI (kg/m^2)	0.22	0.64
	Baseline adiponectin (ug/ml)	509.14	<0.0001
	Time	0.01	0.94
	Age	2.54	0.11
	Gender	2.83	0.09
	Race	0.03	0.86
	Smoking status	0.72	0.39

Note, WC, waist circumference
Reference groups; Time (baseline); Gender (female); Race (White); Smoking status (smokers)

Table 5-5 Relationships between changes in body fat and adiponectin: mixed models

		F vaule	*P* value
Model 1	Body fat Δ (%)	3.97	0.04
	Time	4.59	0.01
	Body fat*time	2.43	0.09
Model 2	Body fat Δ (%)	9.29	0.002
	Time	0.09	0.76
	Baseline body fat (%)	0.00	0.96
	Baseline adipnectin (ug/ml)	532.38	<0.0001
	Age	1.97	0.16
	Gender	2.78	0.09
	Race	0.08	0.78
Model 3	Body fat Δ (%)	9.90	0.002
	Time	0.07	0.79
	Baseline body fat (%)	0.03	0.86
	Baseline adipnectin (ug/ml)	535.68	<0.0001
	Age	1.53	0.21
	Gender	2.35	0.12
	Race	0.11	0.74
	Smoking status	1.41	0.23

Note, Reference groups: Time (baseline); Gender (female); Race (White); Smoking status (non smokers)

Table 5-6 Relationships between changes in fat free mass and adiponectin: mixed models

		F vaule	*P* value
Model 1	FFM Δ (kg)	0.06	0.81
	Time	0.17	0.84
	FFM*time	1.34	0.26
Model 2	FFM Δ (kg)	0.09	0.76
	Time	0.00	0.99
	Baseline FFM (kg)	0.00	0.95
	Baseline adiponectin (ug/ml)	485.01	<0.0001
	Age	1.54	0.21
	Gender	1.02	0.31
	Race	0.21	0.65
Model 3	FFM Δ (kg)	0.05	0.81
	Time	22.21	0.98
	Baseline FFM (kg)	0.01	0.92
	Baseline adiponectin (ug/ml)	480.88	<0.0001
	Age	1.32	0.25
	Gender	1.21	0.27
	Race	0.27	0.60
	Smoking status	0.59	0.44

Note, FFM, fat free mass
Reference groups: Time (baseline); Gender (female); Race (White); Smoking status (non smokers)

Table 5-7 Relationships between changes in physical measures and adiponectin over time: estimates from mixed models

Measures	Estimate (s.e.)	p	Estimate (s.e)	p
Independent variable: changes in weight over time				
Weight Δ (kg)	-0.15 (0.05)	0.002	-0.14 (0.05)	0.003
Time	0.03 (0.42)	0.93	0.04 (0.42)	0.91
Baseline weight (kg)	-0.002 (0.02)	0.90	-0.005 (0.02)	0.79
Baseline adiponectin (ug/ml)	0.95 (0.04)	<0.0001	0.96 (0.04)	<0.0001
Age	-0.06 (0.03)	0.08	-0.05 (0.03)	0.10
Gender	1.32 (0.99)	0.18	1.35 (0.99)	0.17
Race	0.11 (0.75)	0.87	0.15 (0.75)	0.84
Smoking status	-	-	0.75 (0.96)	0.43
Independent variable: changes in BMI over time				
BMI Δ (kg/m^2)	-0.39 (0.13)	0.003	-0.39 (0.13)	0.004
Time	0.02 (0.42)	0.96	0.03 (0.42)	0.94
Baseline BMI (kg/m^2)	-0.02(0.06)	0.70	-0.03 (0.06)	0.64
Baseline adiponectin (ug/ml)	0.95 (0.04)	<0.0001	0.96 (0.04)	<0.0001
Age	-0.05 (0.03)	0.09	-0.05 (0.03)	0.11

Table 5-7 Contd.

Measures	Estimate (s.e.)	*P*	Estimate (s.e)	*P*
Gender	1.52 (0.88)	0.08	1.49 (0.88)	0.09
Race	0.09(0.76)	0.90	0.81 (0.95)	0.39
Smoking status	-	-	-1.69 (2.19)	0.43
Independent variable: change in waist circumference over time				
Waist circumference Δ (cm)	-0.13 (0.05)	0.006	-0.13 (0.05)	0.006
Time 6 months	0.07 (0.42)	0.86	0.08 (0.43)	0.85
\Baseline WC (cm)	0.02 (0.02)	0.39	0.02 (0.03)	0.49
Baseline adiponectin (ug/ml)	0.95 (0.04)	<0.0001	0.95 (0.04)	<0.0001
Age	-0.06 (0.03)	0.07	-0.05 (0.03)	0.10
Gender	0.10 (0.96)	0.30	1.02 (0.96)	0.29
Race	0.45 (0.74)	0.54	0.47 (0.74)	0.52
Smoking status	-	-	-0.60 (0.96)	0.53
Independent variable: change in body fat over time				
Body fat Δ (%)	-0.21 (0.07)	0.002	-0.21 (0.07)	0.002
Time 6 months	-0.13 (0.43)	0.76	1.98 (0.35)	<0.0001
Baseline body fat (%)	-0.003 (0.06)	0.96	-0.01 (0.06)	0.86
Baseline adipnectin (ug/ml)	0.95 (0.04)	<0.0001	0.96 (0.41)	<0.0001

Table 5-7 Contd.

Measures	Estimate (s.e.)	*P*	Estimate (s.e)	*P*
Age	-0.05 (0.03)	0.16	-0.04 (0.03)	0.21
Gender	1.70 (1.02)	0.71	1.57 (1.02)	0.12
Race	0.21 (0.74)	0.78	0.24 (0.74)	0.74
Smoking status	-	-	1.11 (0.94)	0.23
Independent variable: change in fat free mass over time				
Fat free mass Δ (kg)	-0.01 (0.04)	0.76	-0.009 (0.04)	0.81
Time	0.002 (0.43)	0.99	0.007 (0.43)	0.98
Baseline fat free mass (kg)	0.0014 (0.03)	0.95	-0.002 (0.03)	0.92
Baseline adiponectin (ug/ml)	0.96 (0.04)	<0.0001	0.96 (0.04)	<0.0001
Age	0.10 (0.07)	0.14	-0.04 (0.03)	0.25
Gender	1.56 (1.54)	0.52	1.72 (1.56)	0.27
Race	-0.04 (0.03)	0.21	0.40 (0.78)	0.60
Smoking status	-	-	-0.77 (1.00)	0.44

Note, BMI, body mass index; WC, waist circumference
Reference groups used: Time (baseline); Gender (female); Race (White); Smoking status (smokers)

6.0 GENERAL DISCUSSION

6.1 OVERALL SUMMARY

This dissertation, designed as three research papers, aimed to examine relationships between diet, weight loss, insulin resistance and adiponectin levels among overweight/obese adults who were participating in a behavioral intervention for weight loss. Based on the available literature suggesting a protective effect of adiponectin on the development of diabetes and CVD, the premise for the three studies was that a lifestyle intervention may increase adiponectin, which in turn may decrease insulin resistance, and that weight loss during a behavioral intervention may increase adiponectin levels.

The first study compared the effect of a STD-D and a LOV-D on changes in adiponectin levels at six months. This was an ancillary study to the PREFER trial, an 18-month clinical trial of a behavioral weight loss intervention. The same behavioral intervention was delivered to both dietary groups; the only difference was that the LOV-D participants were instructed to eliminate meat, poultry, and fish from their diet. Eligible participants included 143 overweight/obese adults (STD-D = 79; LOV-D = 64) with a mean BMI of 33.7 kg/m^2. The sample was 88% female, 67% White, and 44.2 ± 8.5 years old. Results of this analysis revealed that the adiponectin levels increased significantly in response to both STD-D and LOV-D with no significant differences in the changes of adiponectin levels between the two groups.

One could argue that the null findings might be due to the LOV-D diet group not strictly adhering to the prescribed diet during the study period. However, a comparison of changes in adiponectin levels between the STD-D and the 100% adherent LOV-D groups also showed no statistically significant differences in the changes of total and HMW adiponectin levels between 100% adherent LOV-D and the STD-D groups. Furthermore, we found significant associations between changes in adiponectin levels and weight loss independent of diet types such that the greater the weight loss, the larger was the increase in adiponectin levels. These findings suggest that more than one type of diet can be followed to increase adiponectin levels as long as weight loss is achieved and maintained. Since weight loss at the higher quartile was associated with greater increases in adiponectin levels, a weight loss close to 10% might be necessary for a significant increase in adiponectin levels.

The second study determined whether baseline levels or intervention-associated changes in total and high molecular weight adiponectin levels were associated with insulin resistance at six months. The study design and sample study were the same as in the first study. Analysis of this study revealed that the increase in total adiponectin was inversely associated with the decrease in HOMA independent of baseline weight and weight change suggesting that total adiponectin contributed to improved insulin sensitivity independent of baseline weight and weight loss. However, the significant association between changes in HMW adiponectin level and HOMA score was no longer present after adjustment for weight change.

These findings suggest that a measurement of only total adiponectin might be adequate to examine these relationships and the role of HMW adiponectin level is no more important or at least similar to that of total adiponectin in relation to weight loss and insulin sensitivity. Association of six-month changes in adiponectin and the HOMA score was modest compared to

baseline measures, suggesting that the cross-sectional measure of total adiponectin might be a comparable indicator in relation to measuring insulin sensitivity among overweight or obese adults. Since total adiponectin contributed to better insulin sensitivity, the study highlights the importance of adiponectin in the development of IR and subsequent development of type 2 diabetes.

The third study assessed the longitudinal relationships of weight, waist circumference and body composition with adiponectin level after six and 12 months of participation in a behavioral intervention for weight loss. This was a secondary data analysis of the SMART trial, a 24 month clinical trial of behavioral weight loss intervention. All participants received standard behavioral treatment for weight loss that included calorie, fat and physical activity goals and group intervention sessions that provided strategies and guidance to help achieve a healthful lifestyle. The sample included 133 overweight/obese adults with a mean BMI of 33.4 ± 4.7 kg/m^2 and was 86% female, 81% White, and 45.3 ± 8.8 years old. We measured circulating adiponectin level, weight, waist circumference and body composition at baseline, six and 12 months. Retention at six months was 91% and 85% at 12 months. A longitudinal analysis with adiponectin levels as the dependent variable showed significant increase in adiponectin with weight reduction, waist circumference and percent body fat; while no such association was found with changes in fat free mass and adiponectin levels over time. These findings suggest a negative feedback of obesity on adiponectin and further provide evidence that the production and secretion of adiponectin is modified by the adipose tissue.

Taken together, the findings from these three studies suggest the beneficial effects of the intervention on changes in adiponectin levels, insulin resistance, weight, percent body fat and waist circumference. Overall our results suggest that weight loss is more important than the type

of diet followed in increasing adiponectin levels. Increased adiponectin levels contribute to lowering HOMA scores and the role of HMW adiponectin levels in relation to weight loss and insulin sensitivity is similar to that of total adiponectin. Furthermore, interventions that target long-term weight loss accompanied by reduction in waist circumference, percent body fat enhance adiponectin levels thereby reducing the risk of developing several chronic diseases, such as diabetes and CVD.

6.2 LIMITATIONS

The PREFER and SMART trials provided a unique opportunity to investigate the relationships between diet, weight loss, insulin resistance and adiponectin levels among overweight/obese adults. However, there were some limitations applicable to all three studies that should be considered when interpreting the findings from this dissertation. The dietary measures were assessed with self-reported dietary intake. Additionally, dietary data collected with the 3-day Food Records may not represent dietary intake over six months. The accepted biomarkers for dietary intake are not easily available; therefore, we must rely on self-reported dietary instruments and use what is considered the best approach. The measures of IR included a surrogate marker, HOMA; however, HOMA was derived from the mathematical model and has been shown to be an adequate indicator of IR and has been used in many epidemiological and clinical studies [70, 77]. Body composition was evaluated with BIA, which is not considered the gold standard. However, the measurement of body composition with BIA has been shown to have good accuracy and reliability and has been suggested as a screening tool in clinical practice and in population based research [132]. Another limitation included a small representation of males

as has been observed in other weight-loss trials, which may be due to a lower percentage of men than women trying to lose weight [136]. Most individuals were female, White, and employed full time limiting the generalizability of these findings to populations with these characteristics.

6.3 PUBLIC HEALTH SIGNIFICANCE

Obesity is a chronic disorder that is associated with increased risk for several chronic diseases including diabetes, hypertension, cardiovascular disease and some cancers[89-91, 137, 138]. Although our understanding of CVD and obesity has improved substantially in recent years, CVD still remains the major cause of morbidity and mortality among Americans. A recent National Health Interview Survey showed that the prevalence of overweight/obesity has plateaued; however, it is still at the level of an epidemic and remains a major and significant public health concern[139]. A higher level of adiponectin secreted by adipose tissue itself is suggested to protect against developing diabetes and CVD. Thus, interventions that enhance adiponectin secretion or action may have potential for CVD and diabetes risk reduction

Given the negative associations between elevated adiponectin levels and the multiple indices of obesity, the findings of the three studies emphasize the importance of weight loss as a significant public health preventive measure to enhance adiponectin levels among the studied population. Prevention of disease, death and disabilities related to CVD requires sustained health behavior or lifestyle changes. Even modest sustained health behavior or lifestyle changes can reduce CVD morbidity and mortality substantially. Thus, interventions that target long-term

weight loss that results in reduced waist circumference and percent body fat help enhance adiponectin levels, thereby reducing the risk for the development of diabetes and CVD.

6.4 FUTURE RESEARCH

As obesity, diabetes and CVD are the result of several factors including genetic, environmental and behavioral, a better understanding of adiponectin physiology in relation to obesity, diabetes and CVD could be of value in identifying means to increase adiponectin levels. It is also important to keep in mind that weight and dietary intake had changed simultaneously once the intervention started in both PREFER and SMART trials. Thus, it is difficult to distinguish if observed increased adiponectin levels was due to changes in dietary intake or from the weight loss per se. Future studies examining the effects of dietary composition in weight-stable population are needed to elucidate the role of weight in the relation between the two.

Although we did not observe differences in adiponectin changes in response to both STD-D and LOV-D in the study, we cannot rule out the possibility of specific food groups having positive effects on adiponectin levels. In particular, cross-sectional studies have suggested that increased adiponectin levels are observed with consumption of a high intake of dietary fiber[33, 34, 99, 140], deep yellow and green vegetables[32] and moderate alcohol intake[34]. Longitudinal studies are required to determine further relationships between the food groups and adiponectin levels.

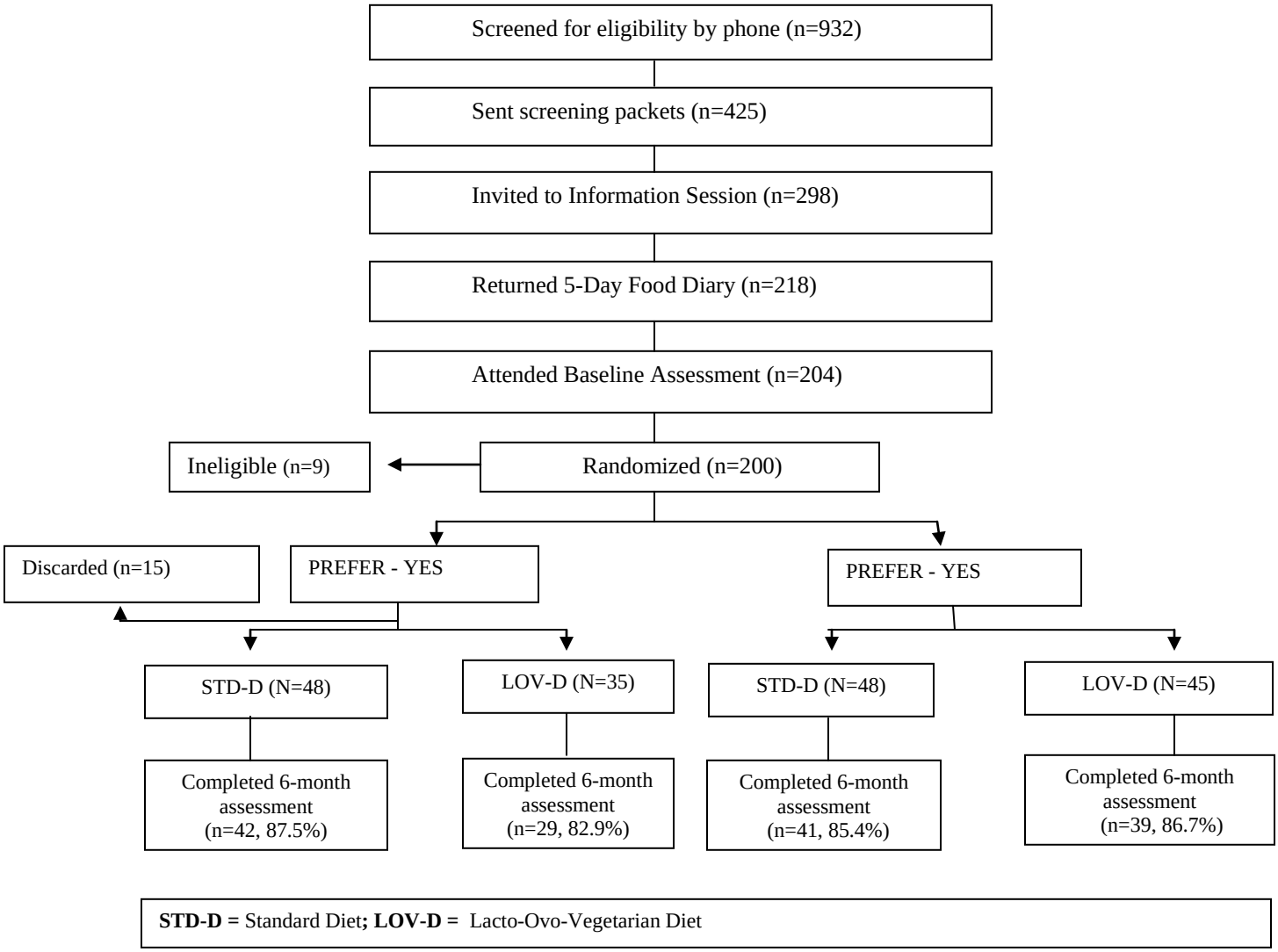
It has been well established that obesity is a major risk factor for the development of diabetes and CVD, however, the mechanism by which adipose tissue contributes to progression of diabetes and CVD is not well understood. Adipose tissue is now recognized as an active

endocrine organ secreting several hormones and a diverse range of other protein factors in recent years[16]. This dissertation was limited to the measurement of only adiponectin levels. It is imperative to further explore the associations of adiponectin with other markers to better understand their contribution to the development of diabetes and CVD.

Prior studies have reported that a higher fat accrual in the abdominal area may have more detrimental effects on diabetes and CVD risk than fat accrued in the lower extremities. Our measure of body composition with BIA did not differentiate between visceral and subcutaneous adipocytes. Therefore, it is important to carry out future studies investigating the changes in regional fat that might help understand how changes in different regional adipose tissue influence changes in adiponectin levels. These studies need to be carried out in populations with diverse ethnic background and wide age range, incorporating both genders allowing the findings to be applicable to a general population.

APPENDIX A

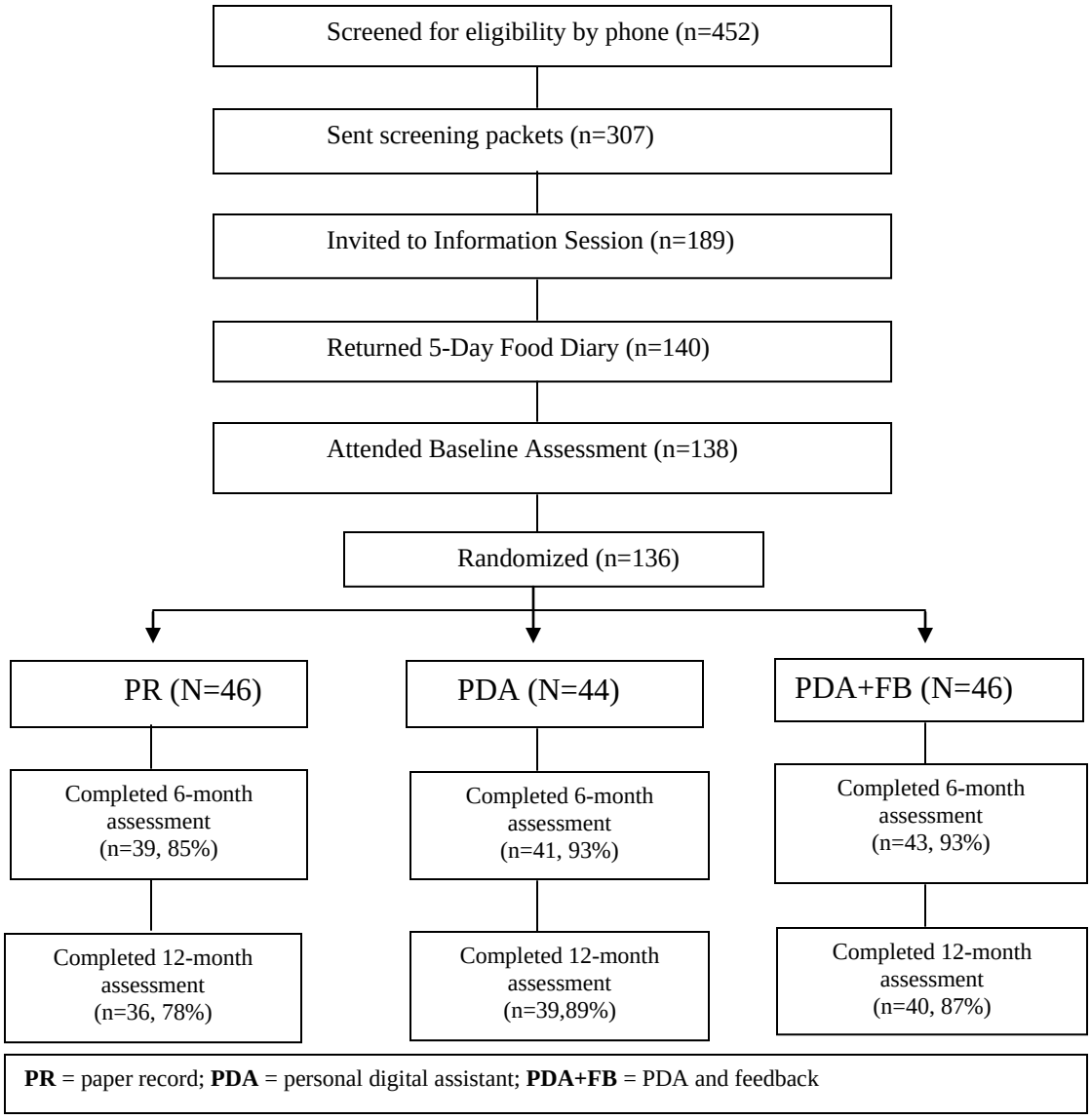
FLOW CHART: PREFER TRIAL



STD-D = Standard Diet**; LOV-D** = Lacto-Ovo-Vegetarian Diet

APPENDIX B

FLOW CHART: SMART TRIAL



BIBLIOGRAPHY

1. Guerre-Millo M. Adipose tissue and adipokines: for better or worse. *Diabetes Metab.* 2004;30(1):13-19.

2. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia.* 2003;46(4):459-469.

3. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol.* 2000;20(6):1595-1599.

4. Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K. Obesity, adiponectin and vascular inflammatory disease. *Curr Opin Lipidol.* 2003;14(6):561-566.

5. Nakashima R, Kamei N, Yamane K, Nakanishi S, Nakashima A, Kohno N. Decreased total and high molecular weight adiponectin are independent risk factors for the development of type 2 diabetes in Japanese-Americans. *J Clin Endocrinol Metab.* 2006;91(10):3873-3877.

6. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab.* 2001;86(5):1930-1935.

7. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease.[see comment]. *Circulation Research.* 2005;96(9):939-949.

8. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation.* 1999;100(25):2473-2476.

9. Lindsay RS, Funahashi T, Hanson RL. Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet.* 2002;360:57-58.

10. Sattar N, Wannamethee G, Sarwar N, Tchernova J, Cherry L, Wallace AM, Danesh J, Whincup PH. Adiponectin and coronary heart disease: a prospective study and meta-analysis. *Circulation.* 2006;114(7):623-629.

11. Schlegel A. Adiponectin and risk of coronary heart disease. *JAMA.* 2004;292(1):40; author reply 40.

12. Schulze MB, Shai I, Rimm EB, Li T, Rifai N, Hu FB. Adiponectin and future coronary heart disease events among men with type 2 diabetes. *Diabetes.* 2005;54(2):534-539.

13. Cavusoglu E, Ruwende C, Chopra V, Yanamadala S, Eng C, Clark LT, Pinsky DJ, Marmur JD. Adiponectin is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction in patients presenting with chest pain. *Eur Heart J.* 2006;27(19):2300-2309.

14. Beltowski J. Adiponectin and resistin--new hormones of white adipose tissue.[see comment]. *Medical Science Monitor.* 2003;9(2):RA55-61.

15. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, Kumada M, Hotta K, Nishida M, Takahashi M, Nakamura T, Shimomura I, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation.* 2002;105(24):2893-2898.

16. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr.* 2004;92(3):347-355.

17. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 1999;257(1):79-83.

18. Chandran M, Phillips SA, Ciaraldi T, Henry RR. Adiponectin: more than just another fat cell hormone? *Diabetes Care.* 2003;26(8):2442-2450.

19. Staiger H, Tschritter O, Machann J, Thamer C, Fritsche A, Maerker E, Schick F, Haring HU, Stumvoll M. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. *Obes Res.* 2003;11(3):368-372.

20. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab.* 2001;86(8):3815-3819.

21. Masaki T, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, Yoshimatsu H. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. *Hepatology.* 2004;40(1):177-184.

22. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, Nagasawa A, Funahashi T, Matsuzawa Y. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation.* 2004;109(17):2046-2049.

23. Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kishida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem.* 2002;277(40):37487-37491.

24. Bobbert T, Rochlitz H, Wegewitz U, Akpulat S, Mai K, Weickert MO, Mohlig M, Pfeiffer AF, Spranger J. Changes of adiponectin oligomer composition by moderate weight reduction. *Diabetes.* 2005;54(9):2712-2719.

25. Fisher FF, Trujillo ME, Hanif W, Barnett AH, McTernan PG, Scherer PE, Kumar S. Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. *Diabetologia.* 2005;48(6):1084-1087.

26. Hara K, Horikoshi M, Yamauchi T, Yago H, Miyazaki O, Ebinuma H, Imai Y, Nagai R, Kadowaki T. Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. *Diabetes Care.* 2006;29(6):1357-1362.

27. Lara-Castro C, Luo N, Wallace P, Klein RL, Garvey WT. Adiponectin multimeric complexes and the metabolic syndrome trait cluster. *Diabetes.* 2006;55(1):249-259.

28. Kobayashi H, Ouchi N, Kihara S, Walsh K, Kumada M, Abe Y, Funahashi T, Matsuzawa Y. Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ Res.* 2004;94(4):e27-31.

29. Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation.* 2001;103(8):1057-1063.

30. Yudkin JS. Adipose tissue, insulin action and vascular disease: inflammatory signals. *Int J Obes Relat Metab Disord.* 2003;27 Suppl 3:S25-28.

31. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol.* 2005;115(5):911-919; quiz 920.

32. Tsukinoki R, Morimoto K, Nakayama K. Association between lifestyle factors and plasma adiponectin levels in Japanese men. *Lipids Health Dis.* 2005;4:27.

33. Mantzoros CS, Williams CJ, Manson JE, Meigs JB, Hu FB. Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women. *Am J Clin Nutr.* 2006;84(2):328-335.

34. Pischon T, Girman CJ, Rifai N, Hotamisligil GS, Rimm EB. Association between dietary factors and plasma adiponectin concentrations in men. *Am J Clin Nutr.* 2005;81(4):780-786.

35. Heidemann C, Hoffmann K, Spranger J, Klipstein-Grobusch K, Mohlig M, Pfeiffer AF, Boeing H. A dietary pattern protective against type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)--Potsdam Study cohort. *Diabetologia.* 2005;48(6):1126-1134.

36. Fargnoli JL, Fung TT, Olenczuk DM, Chamberland JP, Hu FB, Mantzoros CS. Adherence to healthy eating patterns is associated with higher circulating total and high-molecular-weight adiponectin and lower resistin concentrations in women from the Nurses' Health Study. *Am J Clin Nutr.* 2008;88(5):1213-1224.

37. Yannakoulia M, Yiannakouris N, Melistas L, Kontogianni MD, Malagaris I, Mantzoros CS. A dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products and low consumption of refined cereals is positively associated with plasma adiponectin levels in healthy women. *Metabolism.* 2008;57(6):824-830.

38. St-Pierre DH, Faraj M, Karelis AD, Conus F, Henry JF, St-Onge M, Tremblay-Lebeau A, Cianflone K, Rabasa-Lhoret R. Lifestyle behaviours and components of energy balance as independent predictors of ghrelin and adiponectin in young non-obese women. *Diabetes Metab.* 2006;32(2):131-139.

39. Anderlova K, Kremen J, Dolezalova R, Housova J, Haluzikova D, Kunesova M, Haluzik M. The influence of very-low-calorie-diet on serum leptin, soluble leptin receptor, adiponectin and resistin levels in obese women. *Physiol Res.* 2006;55(3):277-283.

40. Garaulet M, Viguerie N, Porubsky S, Klimcakova E, Clement K, Langin D, Stich V. Adiponectin gene expression and plasma values in obese women during very-low-calorie diet. Relationship with cardiovascular risk factors and insulin resistance. *J Clin Endocrinol Metab.* 2004;89(2):756-760.

41. Xydakis AM, Case CC, Jones PH, Hoogeveen RC, Liu MY, Smith EO, Nelson KW, Ballantyne CM. Adiponectin, inflammation, and the expression of the metabolic

syndrome in obese individuals: the impact of rapid weight loss through caloric restriction. *J Clin Endocrinol Metab.* 2004;89(6):2697-2703.

42. Behre CJ, Gummesson A, Jernas M, Lystig TC, Fagerberg B, Carlsson B, Carlsson LM. Dissociation between adipose tissue expression and serum levels of adiponectin during and after diet-induced weight loss in obese subjects with and without the metabolic syndrome. *Metabolism.* 2007;56(8):1022-1028.

43. Dvorakova-Lorenzova A, Suchanek P, Havel PJ, Stavek P, Karasova L, Valenta Z, Tintera J, Poledne R. The decrease in C-reactive protein concentration after diet and physical activity induced weight reduction is associated with changes in plasma lipids, but not interleukin-6 or adiponectin. *Metabolism.* 2006;55(3):359-365.

44. Asayama K, Hayashibe H, Dobashi K, Uchida N, Nakane T, Kodera K, Shirahata A, Taniyama M. Decrease in serum adiponectin level due to obesity and visceral fat accumulation in children. *Obes Res.* 2003;11(9):1072-1079.

45. Bruun JM, Helge JW, Richelsen B, Stallknecht B. Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. *Am J Physiol Endocrinol Metab.* 2006;290(5):E961-967.

46. Polak J, Kovacova Z, Jacek M, Klimcakova E, Kovacikova M, Vitkova M, Kuda O, Sebela M, Samcova E, Stich V. An increase in plasma adiponectin multimeric complexes follows hypocaloric diet-induced weight loss in obese and overweight pre-menopausal women. *Clin Sci (Lond).* 2007;112(11):557-565.

47. Wolfe BE, Jimerson DC, Orlova C, Mantzoros CS. Effect of dieting on plasma leptin, soluble leptin receptor, adiponectin and resistin levels in healthy volunteers. *Clin Endocrinol (Oxf).* 2004;61(3):332-338.

48. Cambuli VM, Musiu MC, Incani M, Paderi M, Serpe R, Marras V, Cossu E, Cavallo MG, Mariotti S, Loche S, Baroni MG. Assessment of adiponectin and leptin as biomarkers of positive metabolic outcomes after lifestyle intervention in overweight and obese children. *J Clin Endocrinol Metab.* 2008;93(8):3051-3057.

49. Arvidsson E, Viguerie N, Andersson I, Verdich C, Langin D, Arner P. Effects of different hypocaloric diets on protein secretion from adipose tissue of obese women. *Diabetes.* 2004;53(8):1966-1971.

50. Cardillo S, Seshadri P, Iqbal N. The effects of a low-carbohydrate versus low-fat diet on adipocytokines in severely obese adults: three-year follow-up of a randomized trial. *Eur Rev Med Pharmacol Sci.* 2006;10(3):99-106.

51. de Luis DA, Aller R, Izaola O, Gonzalez Sagrado M, Bellioo D, Conde R. Effects of a low-fat versus a low-carbohydrate diet on adipocytokines in obese adults. *Horm Res.* 2007;67(6):296-300.

52. Keogh JB, Brinkworth GD, Noakes M, Belobrajdic DP, Buckley JD, Clifton PM. Effects of weight loss from a very-low-carbohydrate diet on endothelial function and markers of cardiovascular disease risk in subjects with abdominal obesity. *Am J Clin Nutr.* 2008;87(3):567-576.

53. Aas AM, Seljeflot I, Torjesen PA, Diep LM, Thorsby PM, Birkeland KI. Blood glucose lowering by means of lifestyle intervention has different effects on adipokines as compared with insulin treatment in subjects with type 2 diabetes. *Diabetologia.* 2006;49(5):872-880.

54. Giannopoulou I, Fernhall B, Carhart R, Weinstock RS, Baynard T, Figueroa A, Kanaley JA. Effects of diet and/or exercise on the adipocytokine and inflammatory cytokine levels of postmenopausal women with type 2 diabetes. *Metabolism.* 2005;54(7):866-875.

55. Weiss EP, Racette SB, Villareal DT, Fontana L, Steger-May K, Schechtman KB, Klein S, Holloszy JO. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. *Am J Clin Nutr.* 2006;84(5):1033-1042.

56. Park TG, Hong HR, Lee J, Kang HS. Lifestyle plus exercise intervention improves metabolic syndrome markers without change in adiponectin in obese girls. *Ann Nutr Metab.* 2007;51(3):197-203.

57. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, Giugliano D. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA.* 2003;289(14):1799-1804.

58. Ng TW, Watts GF, Barrett PH, Rye KA, Chan DC. Effect of weight loss on LDL and HDL kinetics in the metabolic syndrome: associations with changes in plasma retinol-binding protein-4 and adiponectin levels. *Diabetes Care.* 2007;30(11):2945-2950.

59. Rokling-Andersen MH, Reseland JE, Veierod MB, Anderssen SA, Jacobs DR, Jr., Urdal P, Jansson JO, Drevon CA. Effects of long-term exercise and diet intervention on plasma adipokine concentrations. *Am J Clin Nutr.* 2007;86(5):1293-1301.

60. Grunberger G, Jen KL, Artiss JD. The benefits of early intervention in obese diabetic patients with FBCx: a new dietary fibre. *Diabetes Metab Res Rev.* 2007;23(1):56-62.

61. Rosell M, Appleby P, E. S, Key T. Weight gain in 5 years in 21,966 meat-eating, fish-eating, vegetarian, and vegan men and women in EPIC-Oxford. *Int J Obes (London).* 2006;30:1389-1396.

62. Robinson F, Hackett A, Billington D, Stratton G. Changing from a mixed to self-selected vegetarian diet-influence on blood lipids. *J Hum Nutr Diet.* 2002;2002(15):323-329.

63. Phillips F, Hackett A, Stratton G, Billington D. Effect of changing to a self-selected vegetarian diet-influence on blood lipids. *J Hum Nutr Diet.* 2004;15:249-255.

64. Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes.* 2001;50(5):1126-1133.

65. Nakamura Y, Ueshima H, Okuda N, Higashiyama A, Kita Y, Kadowaki T, Okamura T, Murakami Y, Okayama A, Choudhury SR, Rodriguez B, Curb JD, Stamler J. Relation of dietary and other lifestyle traits to difference in serum adiponectin concentration of Japanese in Japan and Hawaii: the INTERLIPID Study. *Am J Clin Nutr.* 2008;88(2):424-430.

66. Steffes MW, Gross MD, Schreiner PJ, Yu X, Hilner JE, Gingerich R, Jacobs DR, Jr. Serum adiponectin in young adults--interactions with central adiposity, circulating levels of glucose, and insulin resistance: the CARDIA study. *Annals of Epidemiology.* 2004;14(7):492-498.

67. Pannacciulli N, Vettor R, Milan G, Granzotto M, Catucci A, Federspil G, De Giacomo P, Giorgino R, De Pergola G. Anorexia nervosa is characterized by increased adiponectin plasma levels and reduced nonoxidative glucose metabolism. *J Clin Endocrinol Metab.* 2003;88(4):1748-1752.

68. Weiss EC, Galuska DA, Khan LK, Serdula MK. Weight-Control Practices Among U.S. Adults, 2001-2002. *Am J Prev Med.* 2006;31(1):18-24.

69. Monzillo LU, Hamdy O, Horton ES, Ledbury S, Mullooly C, Jarema C, Porter S, Ovalle K, Moussa A, Mantzoros CS. Effect of lifestyle modification on adipokine levels in obese subjects with insulin resistance. *Obes Res.* 2003;11(9):1048-1054.

70. Ryan AS, Nicklas BJ, Berman DM, Elahi D. Adiponectin levels do not change with moderate dietary induced weight loss and exercise in obese postmenopausal women. *Int J Obes Relat Metab Disord.* 2003;27(9):1066-1071.

71. Berk ES, Kovera AJ, Boozer CN, Pi-Sunyer FX, Johnson JA, Albu JB. Adiponectin levels during low- and high-fat eucaloric diets in lean and obese women. *Obes Res.* 2005;13(9):1566-1571.

72. Madsen EL, Rissanen A, Bruun JM, Skogstrand K, Tonstad S, Hougaard DM, Richelsen B. Weight loss larger than 10% is needed for general improvement of levels of

circulating adiponectin and markers of inflammation in obese subjects: a 3-year weight loss study. *Eur J Endocrinol.* 2008;158(2):179-187.

73. Abbasi F, Chu JW, Lamendola C, McLaughlin T, Hayden J, Reaven GM, Reaven PD. Discrimination between obesity and insulin resistance in the relationship with adiponectin. *Diabetes.* 2004;53(3):585-590.

74. Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G. Adiponectin and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes.* 2004;53(9):2473-2478.

75. O'Leary VB, Jorett AE, Marchetti CM, Gonzalez F, Phillips SA, Ciaraldi TP, Kirwan JP. Enhanced adiponectin multimer ratio and skeletal muscle adiponectin receptor expression following exercise training and diet in older insulin-resistant adults. *Am J Physiol Endocrinol Metab.* 2007;293(1):E421-427.

76. Frystyk J, Berne C, Berglund L, Jensevik K, Flyvbjerg A, Zethelius B. Serum adiponectin is a predictor of coronary heart disease: a population-based 10-year follow-up study in elderly men. *J Clin Endocrinol Metab.* 2007;92(2):571-576.

77. Hung J, McQuillan BM, Thompson PL, Beilby JP. Circulating adiponectin levels associate with inflammatory markers, insulin resistance and metabolic syndrome independent of obesity. *Int J Obes (Lond).* 2008;32(5):772-779.

78. Rubin DA, McMurray RG, Harrell JS, Hackney AC, Thorpe DE, Haqq AM. The association between insulin resistance and cytokines in adolescents: the role of weight status and exercise. *Metabolism.* 2008;57(5):683-690.

79. Hivert MF, Sullivan LM, Fox CS, Nathan DM, D'Agostino RB, Sr., Wilson PW, Meigs JB. Associations of adiponectin, resistin, and tumor necrosis factor-alpha with insulin resistance. *J Clin Endocrinol Metab.* 2008;93(8):3165-3172.

80. Kowalska I, Straczkowski M, Nikolajuk A, Adamska A, Karczewska-Kupczewska M, Otziomek E, Kinalska I, Gorska M. Insulin resistance, serum adiponectin, and proinflammatory markers in young subjects with the metabolic syndrome. *Metabolism.* 2008;57(11):1539-1544.

81. Heidemann C, Sun Q, van Dam RM, Meigs JB, Zhang C, Tworoger SS, Mantzoros CS, Hu FB. Total and high-molecular-weight adiponectin and resistin in relation to the risk for type 2 diabetes in women. *Ann Intern Med.* 2008;149(5):307-316.

82. Saltevo J, Laakso M, Jokelainen J, Keinanen-Kiukaanniemi S, Kumpusalo E, Vanhala M. Levels of adiponectin, C-reactive protein and interleukin-1 receptor antagonist are associated with insulin sensitivity: a population-based study. *Diabetes Metab Res Rev.* 2008;24(5):378-383.

83. Borges RL, Ribeiro-Filho FF, Carvalho KM, Zanella MT. [Impact of weight loss on adipocytokines, C-reactive protein and insulin sensitivity in hypertensive women with central obesity]. *Arq Bras Cardiol.* 2007;89(6):409-414.

84. Hulver MW, Zheng D, Tanner CJ, Houmard JA, Kraus WE, Slentz CA, Sinha MK, Pories WJ, MacDonald KG, Dohm GL. Adiponectin is not altered with exercise training despite enhanced insulin action. *American Journal of Physiology - Endocrinology & Metabolism.* 2002;283(4):E861-865.

85. Marcell TJ, McAuley KA, Traustadottir T, Reaven PD. Exercise training is not associated with improved levels of C-reactive protein or adiponectin. *Metabolism: Clinical & Experimental.* 2005;54(4):533-541.

86. Yatagai T, Nishida Y, Nagasaka S, Nakamura T, Tokuyama K, Shindo M, Tanaka H, Ishibashi S. Relationship between exercise training-induced increase in insulin sensitivity and adiponectinemia in healthy men. *Endocrine Journal.* 2003;50(2):233-238.

87. Corpeleijn E, Feskens EJ, Jansen EH, Mensink M, Saris WH, Blaak EE. Lifestyle intervention and adipokine levels in subjects at high risk for type 2 diabetes: the Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM). *Diabetes Care.* 2007;30(12):3125-3127.

88. Merl V, Peters A, Oltmanns KM, Kern W, Born J, Fehm HL, Schultes B. Serum adiponectin concentrations during a 72-hour fast in over- and normal-weight humans. *Int J Obes.* 2005;29(8):998-1001.

89. Dal Maso L, Augustin LS, Karalis A, Talamini R, Franceschi S, Trichopoulos D, Mantzoros CS, La Vecchia C. Circulating adiponectin and endometrial cancer risk. *J Clin Endocrinol Metab.* 2004;89(3):1160-1163.

90. Mantzoros C, Petridou E, Dessypris N, Chavelas C, Dalamaga M, Alexe DM, Papadiamantis Y, Markopoulos C, Spanos E, Chrousos G, Trichopoulos D. Adiponectin and breast cancer risk. *J Clin Endocrinol Metab.* 2004;89(3):1102-1107.

91. Wei EK, Giovannucci E, Fuchs CS, Willett WC, Mantzoros CS. Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study. *J Natl Cancer Inst.* 2005;97(22):1688-1694.

92. Faraj M, Havel PJ, Phelis S, Blank D, Sniderman AD, Cianflone K. Plasma acylation-stimulating protein, adiponectin, leptin, and ghrelin before and after weight loss induced by gastric bypass surgery in morbidly obese subjects. *J Clin Endocrinol Metab.* 2003;88(4):1594-1602.

93. Abbasi F, Chang SA, Chu JW, Ciaraldi TP, Lamendola C, McLaughlin T, Reaven GM, Reaven PD. Improvements in insulin resistance with weight loss, in contrast to

rosiglitazone, are not associated with changes in plasma adiponectin or adiponectin multimeric complexes. *Am J Physiol Regul Integr Comp Physiol.* 2006;290(1):R139-144.

94. Stilley CS, Sereika S, Muldoon MF, Ryan CM, Dunbar-Jacob J. Psychological and cognitive function: predictors of adherence with cholesterol lowering treatment. *Ann Behav Med.* 2004;27(2):117-124.

95. Barnard ND, Gloede L, Cohen J, Jenkins DJ, Turner-McGrievy G, Green AA, Ferdowsian H. A low-fat vegan diet elicits greater macronutrient changes, but is comparable in adherence and acceptability, compared with a more conventional diabetes diet among individuals with type 2 diabetes. *J Am Diet Assoc.* 2009;109(2):263-272.

96. Burke LE, Choo J, Music E, Styn MA, Steenkiste AR, Warziski M. PREFER study: a randomized clinical trial testing treatment preference and two dietary options in behavioral weight management-rationale, design and baseline characteristics. *Contemp Clin Trials.* 2006;27:34-48.

97. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O'Brien WL, Bassett DR, Jr., Schmitz KH, Emplaincourt PO, Jacobs DR, Jr., Leon AS. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc.* 2000;32(9 Suppl):S498-504.

98. Qi L, Rimm E, Liu S, Rifai N, Hu FB. Dietary glycemic index, glycemic load, cereal fiber, and plasma adiponectin concentration in diabetic men. *Diabetes Care.* 2005;28(5):1022-1028.

99. Qi L, van Dam RM, Liu S, Franz M, Mantzoros C, Hu FB. Whole-grain, bran, and cereal fiber intakes and markers of systemic inflammation in diabetic women. *Diabetes Care.* 2006;29(2):207-211.

100. Wycherley TP, Brinkworth GD, Keogh JB, Noakes M, Buckley JD, Clifton PM. Long-term effects of weight loss with a very low carbohydrate and low fat diet on vascular function in overweight and obese patients. *J Intern Med.*267(5):452-461.

101. Yeung EH, Appel LJ, Miller ER, 3rd, Kao WH. The Effects of Macronutrient Intake on Total and High-molecular Weight Adiponectin: Results From the OMNI-Heart Trial. *Obesity (Silver Spring).* 2009.

102. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. *Arterioscler Thromb Vasc Biol.* 2004;24(1):29-33.

103. Christiansen T, Paulsen SK, Bruun JM, Ploug T, Pedersen SB, Richelsen B. Diet-induced weight loss and exercise alone and in combination enhance the expression of adiponectin receptors in adipose tissue and skeletal muscle, but only diet-induced weight loss enhanced circulating adiponectin. *J Clin Endocrinol Metab.*95(2):911-919.

104. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med.* 2002;8(11):1288-1295.

105. Mojiminiyi OA, Abdella NA, Al Arouj M, Ben Nakhi A. Adiponectin, insulin resistance and clinical expression of the metabolic syndrome in patients with Type 2 diabetes. *Int J Obes.* 2007;31(2):213-220.

106. Mather KJ, Funahashi T, Matsuzawa Y, Edelstein S, Bray GA, Kahn SE, Crandall J, Marcovina S, Goldstein B, Goldberg R. Adiponectin, change in adiponectin, and progression to diabetes in the Diabetes Prevention Program. *Diabetes.* 2008;57(4):980-986.

107. Salani B, Briatore L, Andraghetti G, Adami GF, Maggi D, Cordera R. High-molecular weight adiponectin isoforms increase after biliopancreatic diversion in obese subjects. *Obesity (Silver Spring).* 2006;14(9):1511-1514.

108. Bluher M, Brennan AM, Kelesidis T, Kratzsch J, Fasshauer M, Kralisch S, Williams CJ, Mantzoros CS. Total and high-molecular weight adiponectin in relation to metabolic variables at baseline and in response to an exercise treatment program: comparative evaluation of three assays. *Diabetes Care.* 2007;30(2):280-285.

109. Haluzik M, Parizkova J, Haluzik MM. Adiponectin and its role in the obesity-induced insulin resistance and related complications. *Physiol Res.* 2004;53(2):123-129.

110. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, Richelsen B. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. *Am J Physiol Endocrinol Metab.* 2003;285(3):E527-533.

111. Milewicz A, Jedrzejuk D, Dunajska K, Lwow F. Waist circumference and serum adiponectin levels in obese and non-obese postmenopausal women. *Maturitas.*65(3):272-275.

112. Park KG, Park KS, Kim MJ, Kim HS, Suh YS, Ahn JD, Park KK, Chang YC, Lee IK. Relationship between serum adiponectin and leptin concentrations and body fat distribution. *Diabetes Res Clin Pract.* 2004;63(2):135-142.

113. Okauchi Y, Kishida K, Funahashi T, Noguchi M, Ogawa T, Ryo M, Okita K, Iwahashi H, Imagawa A, Nakamura T, Matsuzawa Y, Shimomura I. Changes in serum adiponectin concentrations correlate with changes in BMI, waist circumference, and estimated visceral fat area in middle-aged general population. *Diabetes Care.* 2009;32(10):e122.

114. Burke LE, Styn MA, Glanz K, Ewing LJ, Elci OU, Conroy MB, Sereika SM, Acharya SD, Music E, Keating AL, Sevick MA. SMART trial: A randomized clinical trial of self-monitoring in behavioral weight management-design and baseline findings. *Contemp Clin Trials.* 2009;30(6):540-551.

115. Acharya SD, Elci OU, Sereika SM, Music E, Styn MA, Turk MW, Burke LE. Adherence to a behavioral weight loss treatment program enhances weight loss and improvements in biomarkers. *Patient Prefer Adherence.* 2009;3:151-160.

116. Shai I, Schwarzfuchs D, Henkin Y, Shahar DR, Witkow S, Greenberg I, Golan R, Fraser D, Bolotin A, Vardi H, Tangi-Rozental O, Zuk-Ramot R, Sarusi B, Brickner D, Schwartz Z, Sheiner E, Marko R, Katorza E, Thiery J, Fiedler GM, Bluher M, Stumvoll M, Stampfer MJ. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *N Engl J Med.* 2008;359(3):229-241.

117. Dixon JB, Strauss BJ, Laurie C, O'Brien PE. Changes in body composition with weight loss: obese subjects randomized to surgical and medical programs. *Obesity (Silver Spring).* 2007;15(5):1187-1198.

118. Heymsfield SB, Thomas D, Nguyen AM, Peng JZ, Martin C, Shen W, Strauss B, Bosy-Westphal A, Muller MJ. Voluntary weight loss: systematic review of early phase body composition changes. *Obes Rev.*

119. Cassidy A, Skidmore P, Rimm EB, Welch A, Fairweather-Tait S, Skinner J, Burling K, Richards JB, Spector TD, MacGregor AJ. Plasma adiponectin concentrations are associated with body composition and plant-based dietary factors in female twins. *J Nutr.* 2009;139(2):353-358.

120. Yannakoulia M, Yiannakouris N, Bluher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab.* 2003;88(4):1730-1736.

121. Havel PJ. Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes.* 2004;53 Suppl 1:S143-151.

122. Elisha B, Ziai S, Karelis AD, Rakel A, Coderre L, Imbeault P, Rabasa-Lhoret R. Similar associations of total adiponectin and high molecular weight adiponectin with cardio-metabolic risk factors in a population of overweight and obese postmenopausal women: a MONET study. *Horm Metab Res.*42(8):590-594.

123. Sidney S, Lewis CE, Hill JO, Quesenberry CP, Jr., Stamm ER, Scherzinger A, Tolan K, Ettinger B. Association of total and central adiposity measures with fasting insulin in a biracial population of young adults with normal glucose tolerance: the CARDIA study. *Obes Res.* 1999;7(3):265-272.

124. Nakamura Y, Sekikawa A, Kadowaki T, Kadota A, Kadowaki S, Maegawa H, Kita Y, Evans RW, Edmundowicz D, Curb JD, Ueshima H. Visceral and subcutaneous adiposity and adiponectin in middle-aged Japanese men: the ERA JUMP study. *Obesity (Silver Spring).* 2009;17(6):1269-1273.

125. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, Murabito JM, Meigs JB, Cupples LA, D'Agostino RB, Sr., O'Donnell CJ. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation.* 2007;116(1):39-48.

126. Kissebah AH. Intra-abdominal fat: is it a major factor in developing diabetes and coronary artery disease? *Diabetes Res Clin Pract.* 1996;30 Suppl:25-30.

127. Wei M, Gaskill SP, Haffner SM, Stern MP. Waist circumference as the best predictor of noninsulin dependent diabetes mellitus (NIDDM) compared to body mass index, waist/hip ratio and other anthropometric measurements in Mexican Americans--a 7-year prospective study. *Obes Res.* 1997;5(1):16-23.

128. Kanaya AM, Harris T, Goodpaster BH, Tylavsky F, Cummings SR. Adipocytokines attenuate the association between visceral adiposity and diabetes in older adults. *Diabetes Care.* 2004;27(6):1375-1380.

129. Goodpaster BH, Krishnaswami S, Resnick H, Kelley DE, Haggerty C, Harris TB, Schwartz AV, Kritchevsky S, Newman AB. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. *Diabetes Care.* 2003;26(2):372-379.

130. Pou KM, Massaro JM, Hoffmann U, Vasan RS, Maurovich-Horvat P, Larson MG, Keaney JF, Jr., Meigs JB, Lipinska I, Kathiresan S, Murabito JM, O'Donnell CJ, Benjamin EJ, Fox CS. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. *Circulation.* 2007;116(11):1234-1241.

131. Nunez C, Gallagher D, Visser M, Pi-Sunyer FX, Wang Z, Heymsfield SB. Bioimpedance analysis: evaluation of leg-to-leg system based on pressure contact footpad electrodes. *Med Sci Sports Exerc.* 1997;29(4):524-531.

132. Das SK, Roberts SB, Kehayias JJ, Wang J, Hsu LK, Shikora SA, Saltzman E, McCrory MA. Body composition assessment in extreme obesity and after massive weight loss induced by gastric bypass surgery. *Am J Physiol Endocrinol Metab.* 2003;284(6):E1080-1088.

133. Wagner DR, Heyward VH, Kocina PS, Stolarczyk LM, Wilson WL. Predictive accuracy of BIA equations for estimating fat-free mass of black men. *Med Sci Sports Exerc.* 1997;29(7):969-974.

134. Burke LE, Hudson AG, Warziski MT, Styn MA, Music E, Elci OU, Sereika SM. Effects of a vegetarian diet and treatment preference on biochemical and dietary variables in overweight and obese adults: a randomized clinical trial. *Am J Clin Nutr*. 2007;86(3):588-596.

135. Gardner CD, Kiazand A, Alhassan S, Kim S, Stafford RS, Balise RR, Kraemer HC, King AC. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. *JAMA.* 2007;297(9):969-977.

136. Tate DF, Jackvony EH, Wing RR. Effects of Internet behavioral counseling on weight loss in adults at risk for type 2 diabetes: a randomized trial. *JAMA.* 2003;289(14):1833-1836.

137. Mokdad AH, Ford ES, Bowman BA. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA.* 2001;289(1):76-79.

138. Yusuf S, Hawken S, Ounpuu S. Obesity and the risk of myocardial infarction in 27 000 participants from 52 countries: a case-control study. *Lancet.* 2005;2005(9497):1640-1649.

139. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and Trends in Obesity Among US Adults, 1999-2008. *JAMA.* 2010;303(3):235-241.

140. Qi L, Meigs JB, Liu S, Manson JE, Mantzoros C, Hu FB. Dietary fibers and glycemic load, obesity, and plasma adiponectin levels in women with type 2 diabetes. *Diabetes Care.* 2006;29(7):1501-1505.