ASSOCIATIONS BETWEEN WEIGHT LOSS AND REGAIN, CYTOKINE CONCENTRATION, AND INSULIN RESISTANCE AMONG OVERWEIGHT/OBESE ADULTS

by

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ABSTRACT

Obesity is a problem of great public health significance, with over one-third of individuals in the U.S. being obese; it is also associated with an increased risk for cardiometabolic diseases. Abnormal cytokine secretion of pro-inflammatory (IL-6 and TNF-α) and anti-inflammatory (adiponectin and IL-10) cytokines is a hallmark of obesity, linking it to the development of insulin resistance (IR). Weight maintenance after intentional weight loss is difficult to achieve, and individuals often regain weight, entering into a pattern of weight cycling. Little is known on the associations between weight cycling, cytokines, and IR. This dissertation, comprising three research papers, aimed to examine these associations among non-diabetic, overweight/obese adults (N=66) enrolled in the Self-Monitoring And Recording using Technology (SMART) Trial, a 24-month clinical trial of behavioral weight loss treatment.

The first paper examined patterns of weight loss and regain and its effect on pro- and anti-inflammatory cytokines from baseline to 24 months. An interaction between gender and percent change in weight on percent change in adiponectin over time was detected [b(se)=0.9(0.2), p=.0003]. There was an association with increases in IL-6 [b(se)=0.9(0.3), p=.001]. The second paper examined patterns of weight loss and regain and their effect on metabolic measures from baseline to 24 months. Weight change was positively associated with changes in insulin [b(se)=0.5(0.1), p≤.0001] and HOMA-IR [b(se)=0.8(0.2), p≤.0001] over time.

The third paper examined polymorphisms in genes encoding IL-6, TNF-α, adiponectin, and IL-10 and their association with cytokine concentration and IR. C allele carriers in *IL-10* polymorphism rs1800896 had higher HOMA-IR compared to TT carriers [b(se)=1.0(0.4), p=0.02]. Variant allele carriers in *IL-10* polymorphisms rs1800871 and rs1800872 had lower HOMA-IR compared to individuals homozygous for the wild type allele [for both polymorphisms: b(se)=-1.2(0.4), p=.01]. There was a significant within-group decrease in HOMA-IR from baseline to 24 months among individuals with the rs1800872 GG genotype but not for T allele carriers.

These findings reveal weight loss to be an important tool in reducing inflammation and improving insulin sensitivity; however, weight regain can attenuate these improvements. Moreover, the association between *IL-10* polymorphisms and IR suggests that cytokine genes play a role in metabolic outcomes.

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PREFACE

I would like to first thank my Lord and Savior Jesus Christ for helping me to make it through. Philippians 1:6 says "…He who has begun a good work in you will complete it until the day of Jesus Christ," and He has certainly been faithful to what He has promised me.

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1.0 DISSERTATION INTRODUCTION

Obesity, defined as a body mass index (BMI) of ≥ 30 kg/m^2, is one of the most pressing public health issues, with over one-third of adults in the U.S. obese[1]. Moreover, type 2 diabetes, a common complication of obesity, affects approximately 26 million adults in the U.S.[2]; indeed, large epidemiologic cohort studies have found a strong independent association between obesity and the development of type 2 diabetes[3]. Insulin resistance (IR), in which glucose uptake by the peripheral tissues targeted by insulin is reduced, contributes to the development of type 2 diabetes[4]. IR is present in obesity and is considered a mediator in the obesity-type 2 diabetes pathway, thus increasing the risk of developing atherosclerosis.

Lifestyle modification, consisting of a reduction in energy intake, increase in energy expenditure, and behavioral strategies to support these changes, is the cornerstone of weight loss treatment and ultimately the prevention of type 2 diabetes [5]and cardiovascular disease (CVD)[6,7]. Research has shown that even modest weight loss (5-10% of initial weight) is associated with improvements in a number of obesity-related comorbidities, including hypertension, dyslipidemia, and diabetes[8]. Results from the National Health and Nutrition Examination Survey found that about 57% of women and 40% of men pursued weight control methods[9]; these methods often produce successful weight loss for the short term[10]. However, long-term weight maintenance after intentional weight loss is difficult to achieve[10], and weight regain is common, with up to 80% of those who lost at least 10% of their body weight failing to maintain this

weight for over one year[11]. This failure to maintain a healthy weight can lead to increased risk for the development of type 2 diabetes.

A potential mechanism in the pathway between obesity and IR is inflammation. Adipose tissue (AT) is an active endocrine organ capable of producing a diverse group of substances, including pro- and anti-inflammatory cytokines[12]. Interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) are two widely researched pro-inflammatory cytokines[13] that are implicated in the development of IR. IL-6 is elevated in obesity[14], and is associated with hyperglycemia[15]. Likewise, excess adipose tissue produces an increased concentration of TNF-α[3], which can impair insulin receptor signaling[16].

In contrast, adiponectin and IL-10 are anti-inflammatory cytokines, and decreased serum levels of these cytokines are associated with obesity and the development of IR. Adiponectin is known to have a protective effect against atherosclerosis[3]. Plasma levels of adiponectin positively correlate with insulin sensitivity (IS)[17], and it is thought that adiponectin accelerates β oxidation of free fatty acids in skeletal muscle, thus improving the peripheral action of insulin[18]. IL-10 is known to have anti-atherogenic properties[19] through the inhibition of a number of immune and inflammatory responses[20]. However, little is known about the association between IL-10 and insulin action[21], although it has been shown to be negatively correlated with fasting plasma glucose[22] and positively correlated with IS[21,22].

Obesity and IR are complex disorders that result not only from environmental and behavioral influences, but also from genetic influences[15,23]. Single-nucleotide polymorphisms (SNPs) in both pro- and anti-inflammatory cytokine genes have been well studied, but their association with obesity, weight regulation, and IR has not been clear[24]. The IL-6 -174 G>C polymorphism has been associated with BMI[25] and fasting insulin[26]; however, data on the role of

this polymorphism in the pathogenesis of obesity and type 2 diabetes is mixed[27]. The effects of variants in TNF-α are equivocal[28], but have been linked to higher glucose levels[29], increased risk for type 2 diabetes[30], less weight loss in bariatric surgery patients[31], and increased waist circumference in women[32].

Variants in adiponectin have been associated with increased risk for obesity, although a recent meta-analysis revealed that the association between adiponectin SNPs and weight is inconsistent[24]. A meta-analysis on adiponectin variants and its role in IR found that some SNPs modulated the risk for IR while others did not[33]. IL-10 SNPs have been linked to BMI[34,35] and waist circumference[35]. The association between IL-10 polymorphisms and insulin indices is understudied in the literature, and the results have been mixed[36]. Some studies have found an association with fasting glucose and HOMA-IR[34,37], while others show a lack of association with glucose[35] or the metabolic syndrome[38].

SNPs in cytokine genes have been shown to be associated with body weight, cytokine concentration, and IR. Therefore, it is possible that SNPs in cytokine genes may modify the association between weight and cytokine concentration, as well as weight and IR. Specifically, individuals carrying a particular allele and are obese may have higher HOMA levels compared to individuals of the alternative allele[39], and weight loss may have a greater effect on cytokine concentration and HOMA for carriers of the variant allele compared to wild type allele carriers[40]. Thus it is plausible that cytokine genes can interact with weight change and other genes to affect IR.

1.1 SPECIFIC AIMS

There is evidence that weight loss is effective at reducing inflammation and improving insulin action, and may be influenced by genetic makeup. However, the majority of studies have focused on individuals categorized as having class III obesity (BMI ≥40 kg/m^2) who have lost an extreme amount of weight through bariatric surgery[41]; results from intervention studies have been mixed. In addition, the effect that weight regain has on inflammation and IR, as well as the role that cytokine SNPs play in weight regain is underexplored in the literature. Given the role of obesity in the development of inflammation and type 2 diabetes, the fact that regain after weight loss is common, and the influence of genes on obesity and the development of type 2 diabetes, it is important to explore the interrelationships among weight changes, cytokines, SNPs in cytokine genes, and IR in order to devise strategies to best reduce weight, improve inflammation, and reduce the risk for type 2 diabetes. Therefore, in a group of obese adults who lost and regained at least 10 pounds during a behavioral weight loss intervention, the aims of this dissertation are to:

1. Determine the longitudinal associations between percent weight change and percent change in cytokines (IL-6, TNF-α, adiponectin, and IL-10) from baseline to 24 months.
2. Determine the effects of percent weight change on percent change in insulin indices (fasting plasma insulin and IR [HOMA-IR]) from baseline to 24 months.
3. Explore the association between cytokine genes, cytokine concentration, and IR during a weight loss intervention.

2.0 REVIEW OF THE LITERATURE

2.1 OVERVIEW OF OBESITY

Obesity, defined as having a body mass index (BMI) of ≥30 kg/m^2, is a complex disorder resulting from an interaction among genetic, behavioral, and environmental factors[42-45]. Obesity rates have rapidly increased within the last few decades[45], reaching epidemic proportions[44,46,47]. This increase suggests that it is environmental and behavioral changes, rather than biological changes, that are responsible for the increased rates[44]. Indeed, the current nutritional environment in the U.S. is characterized by excess availability and consumption of energy-dense foods concurrent with an increase in sedentary lifestyle[42,45]. The increased obesity rates have led to an increase in the number of complications, including cardiovascular disease[48], dyslipidemia, the metabolic syndrome[49], and certain cancers[50-52]. Given the combination of these factors, obesity is one of the most pressing public health issues globally[47].

In the U.S., results from the 2009-2010 National Health and Nutrition Examination Survey reveal that the age-adjusted prevalence of obesity is approximately 36%, with rates for men being 35.5% and for women 35.8%[1]. While there are subtle differences in the prevalence of obesity between men and women, there are significantly larger differences between racial/ethnic

groups[1]. The prevalence of class II (defined as BMI of 35-<40 kg/m^2) and class III (defined as BMI of ≥40 kg/m^2) obesity is growing, with about 15% of the population having a BMI ≥35 kg/m^2 [1]. These numbers give a startling picture of the magnitude of the obesity epidemic.

2.2 APPROACHES TO OBESITY TREATMENT

There are three approaches used to treat obesity: bariatric surgery, pharmacotherapy, and standard behavioral therapy (SBT) for weight loss. Studies have shown that bariatric surgery can result in large weight loss that is sustained over time, leading to improvements in cardiometabolic risk factors[53]. However, there are limitations to bariatric surgery, including long-term health effects of the procedure and the fact that it is only available to the extremely obese or the obese with co-morbidities such as type 2 diabetes[53]. Pharmacotherapy such as orlistat and sibutramine are effective in regulating weight, but as with bariatric surgery, they have limitations, such as adverse health effects and contraindication to their use[54]. SBT, which consists of a reduction in energy intake, increased energy expenditure, and behavioral therapy, is the standard approach to weight loss treatment[6]. It has been shown to be effective in the reduction of weight[5]. Despite its effectiveness, individuals tend to regain weight and can be susceptible to weight cycling.

2.2.1 Difficulties with weight loss maintenance and risk for weight cycling

Weight loss is common, with about 57% of women and 40% of men currently attempting to lose weight[9]. Participants who enroll in weight loss studies tend to lose weight quickly, often

in the first 6 months[55], and it has been shown that initial weight loss as low as 5% is enough to prevent or improve cardiometabolic diseases[56]. However, weight maintenance after weight loss has been proven difficult to achieve; only 20% of individuals are successful in long-term weight maintenance[57,58]. In fact, the lack of long-term success in behavioral therapy aimed at weight loss has been reported in the literature for over 30 years[56,59]. Most who intentionally lose weight will regain about 35% of the weight that is lost within a year[56,60] and about 95% within five years[57], thus entering into a pattern of weight loss and regain known as weight cycling. Furthermore, although modest weight loss can improve health outcomes, it is still unclear whether these improvements remain or are attenuated with weight regain[61]. A meta-analysis of weight loss clinical trials with a minimum of 1-year follow-up revealed that the mean 6-month weight loss of subjects based on a review of 80 studies (N=18,199 completers) was achieved in all lifestyle modification groups, with the highest for those assigned to a very-low-energy diet (16%), followed by meal replacements (9.6%), diet plus exercise (8.5%), diet alone (5%), and exercise alone (2.7%). However, weight regain in the long-term (ranging from 12-48 months) was detected in all three groups with the very-low-energy diet group experiencing the largest and most rapid weight regain[62]. The results of the meta-analysis reveal the complex nature of weight maintenance after successful weight loss[56].

2.3 OBESITY AND INSULIN RESISTANCE

One of the most prevalent complications of obesity is the development of type 2 diabetes; currently about 26 million adults in the U.S. are affected by the disease[63]. Uncontrolled type 2

diabetes can lead to a host of health problems, including myocardial infarction, stroke, blindness, and renal failure[64]. A major contributor to the development of type 2 diabetes is insulin resistance (IR), which is defined as the reduction in glucose uptake in adipose and skeletal muscle tissues[4]. Insulin is an essential hormone that increases glucose disposal in skeletal muscle and adipose tissue, while inhibiting gluconeogenesis in liver, with the goal of regulating glucose homeostasis[65]. Insulin also modulates the metabolism of fats[66], targets the heart and vascular endothelium, and helps maintain metabolic and cardiovascular homeostasis[67,68]. Adipose tissue IR contributes to the development of cardiovascular diseases such as atherosclerosis through such mechanisms as increasing the release of free fatty acids into the circulation[69]. It is also believed that IR may predate the development of type 2 diabetes by 10-20 years[70], emphasizing the importance of preventing and correcting this phenomenon in order to prevent future serious medical complications.

A wealth of empirical studies have examined the association between obesity and IR, although this association is not definite[71]. Epidemiological studies have found a positive association between IR and various measures of adiposity, including body weight, waist circumference, and fat mass[72]. In addition, major population-based studies, such as the Diabetes Prevention Program[5,73], have shown that lifestyle modification resulting in weight loss is associated with changes in IR. It is still unclear what the effects of weight regain are on IR since few studies have examined this relationship.

Patterns of weight loss and regain, or weight cycling, have been reported to be associated with insulin concentration and/or IR, but the association is not conclusive[74]. Some studies found a significant association[75], while others have not[76,77]. The inconclusiveness is likely due to the challenges associated with finding the appropriate definition for weight cycling, and limitations

of studies that do investigate this phenomenon, such as inclusion of both unintentional and intentional weight loss, cross-sectional rather than longitudinal studies[78], and lack of detail regarding amount and timing of weight regain[76,78]. Clearly, this is an important phenomenon in terms of weight maintenance and overall health, and needs to be explored further in the literature.

2.3.1 Adipose Tissue Pathology and Insulin Resistance

In response to obesity, white adipose tissue rapidly expands through adipocyte hypertrophy and hyperplasia in order to maintain whole-body energy homeostasis[79]. This expansion can lead to two mechanisms in the development of metabolic dysfunction: 1) the release of free fatty acids into the circulation, eventually leading to ectopic fat deposition[80]; and 2) a reduction in blood flow that causes an inflammatory state, possibly due to hypoxia and adipocyte necrosis[81].

2.3.1.1 Adipose Tissue Expansion and the Overflow Hypothesis

Adipose tissue normally functions as a buffer by suppressing the release of free fatty acids into circulation and increasing triglyceride clearance[47]. However, in obesity, adipose tissue becomes overloaded with triglycerides, decreasing the buffer capacity for fat storage in adipocytes[82]. Thus, the "overflow hypothesis" states that there is limited capacity for adipose tissue to store fuel; therefore, long-term energy intake can result in the overflow of excess lipids to non-adipose tissue in places such as the liver and muscle, disrupting their functions[83] and causing IR[47]. It has been shown that adipocyte hypertrophy is an independent risk marker of

IR[84]. Free fatty acids can also activate the inflammatory response by increasing local extracellular lipid concentrations, leading to macrophage accumulation[79,85].

2.3.1.2 Inflammatory Processes in Adipose Tissue Expansion

Obesity is characterized by chronic low-grade inflammation, and this inflammatory state has a critical role in the pathogenesis of metabolic dysfunction[81]. The pathological expansion of white adipose tissue is characterized by the rapid growth of fat pad, leading to hypoxia[86], macrophage infiltration, up-regulation of pro-inflammatory markers, and down-regulation of anti-inflammatory markers[79,81]. Therefore, the endocrine function of white adipose tissue depends on secretory processes of both adipocytes and the stromal vascular cells[87], particularly macrophages, and obesity can modify the secretory status of white adipose tissue[81]. There are two types of macrophages that are part of white adipose tissue: classically activated (M1) and alternatively activated (M2) macrophages. M2 macrophages are considered the anti-inflammatory phenotype and predominate in white adipose tissue of lean individuals, while M1 macrophages are pro-inflammatory in nature and predominate in white adipose tissue of obese individuals[88]. Although the mechanisms are not completely understood, obesity leads to immune imbalance in white adipose tissue, with a shift from M2 anti-inflammatory macrophages to the M1 type[89]. It has been shown that weight loss and maintenance result in a reduction of macrophages and a decrease in the pro-inflammatory profile in obese individuals[90].

2.3.2 Weight Loss Interventions Investigating the Impact of Weight Change on Insulin Resistance

The effects of weight loss from SBT on IR are outlined in 13 studies[53,61,72,91-100] (Table 2-1). The interventions ranged in duration from four weeks to two years. Only four studies[53,91,93,97] had interventions lasting a year or longer, highlighting the lack of long-term weight loss interventions reported in the literature. Six[53,61,72,91,94,99] of the thirteen studies had more than one follow-up time point. Of these six studies, only one[53] reported no regain in either group. The amount of weight regained from period of weight loss to the end of the study ranged from 1.5% to 31.4%. Overall, the majority of studies found that weight loss decreased HOMA-IR (homeostatic model assessment-insulin resistance), regardless of the amount of weight lost. Four studies[61,72,91,99] revealed that when weight was regained at follow-up, there was some attenuation of improvement in HOMA-IR that were observed after weight loss. However, HOMA-IR was still lower at follow-up compared to baseline, suggesting that a certain amount of weight loss may have some long-term protective effects for IR.

These studies reveal that SBT for weight loss has a positive effect on reducing IR. Despite the short-term nature of most of the interventions and variations in sample size, populations studied, and intervention components, it is clear that weight change has a positive effect on IR. However, more attention needs to be given to the role of weight regain in these factors.

2.4 CYTOKINES, OBESITY, AND INSULIN RESISTANCE

The link between obesity and a chronic low-grade inflammatory state has been established from epidemiological studies dating back to the 1950s, although the mechanisms behind this association have not been fully elucidated[89]. One potential mechanism is the dysregulation in the balance between pro- and anti-inflammatory cytokines, leading to chronic low-grade systemic inflammation that contributes to the development of cardiometabolic disorders[88]. Cytokines are involved in a variety of biological processes, including lipid and carbohydrate metabolism, angiogenesis, immune response, adipocyte proliferation and differentiation, and blood pressure regulation[88]. The literature has shown cytokine concentrations to be altered in obesity, and weight loss generally results in improvement in cytokine levels[90]. However, very few studies have examined the effect of weight regain and weight cycling on obesity-associated inflammation; therefore, a significant gap in the scientific literature remains[74]. The paragraphs that follow provide an overview of four cytokines that are the focus of the proposed dissertation study: IL-6, TNF-α, adiponectin, and IL-10 (Table 2-2).

2.4.1 IL-6

IL-6 is a pleiotropic cytokine that has both pro- and anti-inflammatory properties[15], depending partly on the tissues that are involved[101]. For example, in skeletal muscle, IL-6 increases glucose metabolism[102], whereas opposite effects are observed involving adipose tissue[103]. IL-6 has a variety of functions, including inducing hepatic C-reactive protein production, a marker of cardiovascular disease[104]; increasing TNF-α secretion; and regulating

hematopoiesis and host defense mechanisms[105]. IL-6 has been implicated in the development of atherosclerosis, as IL-6 mRNA is present in atherosclerotic arteries at a rate that is 10-40 times higher than in non-atherosclerotic arteries[106]. It is produced by several cell types, including endothelial, skeletal and smooth muscle, adipocytes, monocytes, and fibroblasts[107]. About 15% to 35% of the total concentration of IL-6 originates from adipose tissue[108], of which the majority comes not from mature adipocytes but from cells representing the stromal vascular fraction[107].

Population-based studies have shown that IL-6 is elevated in individuals who are obese, and IL-6 plays a key role in the interaction between obesity, inflammation, and cardiovascular disease[109-111]. For example, among adult women, those who were obese had a significantly higher concentration of IL-6 compared with women who were lean[112]. Another study of overweight and normal weight boys found IL-6 levels to be significantly higher in overweight compared with normal-weight boys[113]. The role that weight loss plays in reducing IL-6 levels is controversial and may be dependent on the amount and mechanism of weight loss. For example, it has been shown that IL-6 concentrations improved with an 8% weight loss in individuals on a low-calorie, low-to-moderate fat intake diet[114], but that a 3% weight loss was not enough to provide significant improvements in IL-6 concentration among overweight individuals on a high-fat, low-calorie diet[115]. The disparate outcomes may be due to differences in the duration of the weight loss intervention as well as the intervention itself. In addition, it has been shown that exercise-induced weight loss did not have a significant impact on IL-6 concentration but that diet-induced weight loss did[116]. Only one study investigated the association between weight regain and IL-6 among individuals who intentionally lost weight as part of a 6-month behavioral intervention[61], and investigators did not find a significant change in IL-6 when weight was regained at 12 months.

The association between IL-6 and obesity has been consistently shown in the literature; however, the association between IL-6 and IR is controversial[107]. There is some debate on whether IL-6 improves insulin sensitivity, due to its release into circulation post-exercise and the fact that this period is the time when insulin action is enhanced, or whether IL-6 promotes IR[117]. Nevertheless, studies have shown IL-6 to contribute to the development of IR by promoting lipolysis in adipocytes, increasing hepatic fatty acid synthesis, and increasing plasma triglyceride concentration via inhibiting lipoprotein lipase activity in AT[118]. It also has been shown that IL-6 decreases the action of insulin[15]; one study found a significant positive correlation between plasma insulin concentration and IL-6 in both diabetic and non-diabetic obese women and healthy lean women[119]. In a study of 142 men and women with varying levels of obesity, insulin sensitivity, and glucose tolerance, there was a dose-response associated with plasma concentrations of IL-6, with those having type 2 diabetes with the highest concentration of IL-6, followed by those with impaired glucose tolerance, and those with normal glucose tolerance having the lowest concentration of IL-6[22]. A large population-based study also found IL-6 to be an independent risk factor for the development of type 2 diabetes[120].

2.4.2 TNF-α

TNF-α is a pro-inflammatory cytokine that is primarily derived from monocytes and macrophages[86,107]. It has a wide range of pro-inflammatory properties[13,121], and its primary role is in stimulating the acute phase of inflammation[122]. TNF-α has been shown to be overexpressed in obesity; it has been found to be produced up to 7.5 times more in the adipose tissue of obese compared to non-obese individuals[123]. Therefore, it is considered an important mediator in the

association between obesity and IR[16]. Epidemiological studies have found TNF-α to be positively associated with BMI[124]. Obese individuals have been shown to have higher concentrations of TNF-α compared to non-obese individuals[125]. However, there does not appear to be a difference in expression and production between subcutaneous and visceral fat[126]. It has been shown that weight loss improves inflammation[127], but the role of weight loss on improving TNF-α concentration is inconsistent. Some studies have shown weight loss to reduce concentrations of TNF-α[128,129], while other studies have not[61,130,131]. It is important to note that one of the studies[129] that found a significant decrease in TNF-α after weight loss had on average, a 15% reduction in weight, whereas the other studies[61,130,131] had an average weight reduction between 3% and 7%. This suggests that amount of weight loss may determine cytokine concentration, but this is not definite. There are limitations to these studies, in particular, the short duration which inhibited the ability to investigate the effect of patterns of weight loss and regain on TNF-α. Only one study investigated the association between weight regain and TNF-α among individuals who intentionally lost weight as part of a behavioral intervention[61], and investigators did not find TNF-α concentrations to significantly change during the weight regain period.

TNF-α has been known to interfere with insulin action, and although the mechanisms behind this are not completely clear, it is thought that it inhibits tyrosine kinase activity of the insulin receptor[103,132], which is crucial in the binding of insulin to its receptor[16]. Obese mouse models lacking TNF-α function have shown improved insulin sensitivity[133]. Since TNF-α has a role in insulin action, it is plausible that TNF-α is also associated with the development of IR. Epidemiological studies have investigated this association, but have produced inconsistent

results[69,124]. TNF-α has also been known to affect lipid metabolism[121] and is positively correlated with triglyceride levels, increasing the risk of coronary heart disease[134].

2.4.3 Adiponectin

Adiponectin, also known as ACRP30, AdipoQ, GBP28, and apM1, is a cytokine highly expressed in adipose tissue[107]. Adiponectin has multiple functions, including regulating insulin sensitivity, energy homeostasis, and tissue remodeling[111]. Adiponectin is a particularly interesting cytokine in that, unlike others, it is reduced in obesity, and reduced levels are associated with the development of IR, type 2 diabetes, and coronary artery disease[13]. There is also a strong correlation between high concentrations of adiponectin and insulin sensitivity[107]. Other cardioprotective properties of adiponectin include the modulation of TNF-α inflammatory response, where it reduces secretion of TNF-α from macrophages[135], and the up-regulation of IL-10, leading to anti-inflammatory activity[136]. Adiponectin also inhibits monocyte adhesion to endothelial cells, preventing atherosclerotic plaque formation[111].

Epidemiological studies have shown adiponectin to be associated with BMI and the development of IR in various populations. In a large study of lean Japanese men and women without severe illness, adiponectin was negatively correlated with BMI, fasting plasma glucose, insulin levels, and IR[137]. Weight loss has been shown to increase adiponectin levels in some studies[138] but not others[139,140]. These differences are likely due to differences in study participants (e.g. both male and female participants vs. female only) and duration of the weight loss intervention. Also, there may be conflicting results regarding the amount of weight loss that is adequate to produce desired changes in adiponectin. For example, a study among participants

who lost 10% or more of their initial body weight showed improvements in adiponectin[138], while other studies among participants who lost <10% of their initial body weight showed no improvements in adiponectin[141,142]. Only three studies have investigated the association between weight regain and adiponectin among individuals who intentionally lost weight as part of an intervention[61,143,144]. Two studies showed increases in adiponectin despite weight regain[61,144], while the other one reported no significant association between weight regain and adiponectin[143]. It is possible that the differences between these three studies have to do with study design. The studies that found increases in adiponectin with weight regain included multiple time points, whereas the study that did not report a significant association examined weight regain in the context of a single event that occurred during a specific time period (a "weight regain period").

2.4.4 IL-10

IL-10 is an anti-inflammatory cytokine that is primarily produced by M2 macrophages and Th2 lymphocytes[145]. Its anti-inflammatory nature is due in part by inhibiting the production of several pro-inflammatory cytokines such as IL-6 and TNF-α[89]. Another atherogenic property of IL-10 includes the prevention of atherosclerotic lesion development[146] and stability[147]. In the area of obesity, lifestyle modification, and weight loss research, IL-10 is an extremely understudied cytokine. A great majority of the studies on IL-10 have been conducted in non-humans; if they involved humans, they were bariatric surgery studies, or involved participants with acute and/or allergic inflammatory conditions. Therefore its expression in obesity is rather conflicting where it has been shown to be either increased[148] or decreased[149].

Very few studies have reported examining the effect of weight loss on IL-10 concentration. One weight loss study in which Korean participants lost an average of 7% of their initial body weight over 12 weeks, circulating IL-10 levels significantly increased[150]. However, another study of U.S. gastric bypass surgery patients found that even with a 22% decrease in weight at 12 weeks post-surgery, there were no significant changes in IL-10 concentration[151]. The disparate outcomes may be due to patient characteristics (e.g. differences in racial groups), or to sample size, as the former study had 78 participants whereas the latter study only had 15. It may also reveal the potential differences in outcomes based on method of weight loss. Only one study investigated the association between weight regain and IL-10 among individuals who participated in a weight loss intervention[61]. A significant change in IL-10 with weight regain was not observed.

The literature on the association between IL-10 and IR is just as obscure. It is likely that IL-10 is associated with IR via the suppression of pro-inflammatory cytokines that interfere with insulin signaling such as TNF-α[152]. However, there is variability in the literature in the association between IL-10 and IR. For example, IL-10 was not shown to be associated with HOMA-IR in African-American, overweight/obese participants[153]. In contrast, a study conducted in Caucasian, slightly overweight (average BMI=26 kg/m^2) participants found IL-10 to be positively associated with insulin sensitivity even after controlling for BMI[21].

2.5 WEIGHT LOSS INTERVENTIONS INVESTIGATING THE IMPACT OF WEIGHT CHANGE ON CYTOKINES AMONG OVERWEIGHT/OBESE ADULTS

The effects of SBT for weight loss on cytokines are outlined in 16 studies[61,92,129-131,138-141,149,154-159] (Table 3). The interventions ranged in duration from 4 weeks to 2 years, although the great majority of the interventions were 6 months or shorter. Overall, there were some mixed results regarding improvement in cytokines after intervention. For example, out of the 12 studies that investigated adiponectin, five found a significant increase in concentration post-intervention[138,139,155-157] while seven did not[61,130,131,141,149,154,159]. Ten studies investigated the effects of a weight loss intervention on IL-6 concentration. Of these, intervention results showed a significant decrease in IL-6 in six studies[92,129,130,139,140,158]. Seven studies investigated the effects of weight loss intervention on TNF-α[61,129-131,156,157,159]. Overall, none of the studies showed a major impact of weight change on TNF-α. However, one study[130] did find a significant decrease in TNF-α post-intervention in those who had impaired glucose tolerance, but not in those with normal glucose tolerance or type 2 diabetes. This suggests that weight loss interventions may be beneficial in reducing TNF-α in those with pre-diabetes, but may not be beneficial in reducing TNF-α concentration in healthy individuals or in individuals with overt diabetes. Lastly, only three studies investigated the effects of a weight loss intervention on IL-10[61,149,156]. In all three studies, there were no significant changes in IL-10 that corresponded with changes in weight.

The lack of significant results for some of the cytokines as well as inconsistency in results between the studies points to a number of limitations of these studies. First, the majority of these studies only collect data at one time point; therefore, the fluctuations in weight and

cytokine concentrations are not captured. Second, the majority of these studies have total sample sizes smaller than 50, with two or more comparison groups of approximately 10 or so individuals. These small samples may lead to inaccurate results. Finally, the majority of these studies had brief (e.g. 4 weeks) short-term interventions, which may not have been long enough to produce the desired results. Additional limitations included variability in the intervention components and differences in the observed weight loss, which may have contributed to inconsistent outcomes. It is interesting to note that the intervention studies that had the most weight loss produced the most improvement in some of the cytokines. For example, in one study where the mean percent weight loss post-intervention was 15% and 3% in the treatment and control groups, respectively, the treatment group had greater improvements in adiponectin and IL-6 compared to the control group[139].

2.6 CYTOKINE GENE POLYMORPHISMS AND INSULIN RESISTANCE

Cytokines play a role in the pathogenesis of IR, and single-nucleotide polymorphisms (SNPs) in cytokine genes may be related to the predisposition to and/or different clinical features or outcomes of IR[160]. Thus, SNPs in cytokine genes could partly explain the variability in cytokine production, and the greater predisposition to certain health outcomes which are mediated by changes in cytokine production[161]. Table 4 outlines eight SNPs in the genes encoding IL-6, TNF-α, adiponectin, and IL-10, which are also discussed in depth below.

2.6.1 IL-6

The *IL-6* gene is located on chromosome 7p21[162], and has been linked to the development of type 2 diabetes[163]. The rs1800795 SNP is a G-to-C substitution at position -174 in the promoter region of the *IL-6* gene, and is the most commonly studied *IL-6* SNP[164]. There is evidence that this SNP represents a functional change involved in transcription of the IL-6 gene, thus making it a risk factor for a number of diseases[165,166]. Indeed, many studies have revealed this SNP to be associated with health outcomes such as rheumatoid arthritis[167] and coronary heart disease[168]. Studies have also shown the C variant allele of rs1800795 to be associated with higher IL-6 levels[26,169] and BMI[169], particularly although this association is controversial.

The association between rs1800795 and IR is also controversial, with the literature revealing mixed results. For example, a study among Swedish Caucasian males without obesity and diabetes found that those who had the GG genotype had lower HOMA-IR compared to C allele carriers[170]. In contrast, a study in Turkish participants with and without polycystic ovary syndrome (PCOS) found no association between rs1800795 genotypes and HOMA-IR in both those with PCOS and healthy controls[171]. These results are echoed by a study including Italian, obese, non-diabetic men and women[172], and by a study that included lean and obese, non-diabetic Indian women[173].

Only a few studies have examined the interaction between weight indices, cytokine concentration, and cytokine variants in relation to IR[39,174,175]. A study among Italian men and women with and without diabetes found C allele carriers to have a significant association between IL-6 and IR in those with type 2 diabetes. This same association was not observed among participants with the GG genotype who were also diabetic, or among those without

diabetes, regardless of their genotype[174]. Two other studies found BMI to significantly modify the association between rs1800795 and IR, with higher BMI being associated with higher HOMA-IR in those who were of the CC genotype compared to G allele carriers[39,175]. No studies have investigated the interaction between rs1800795 and changes in weight on changes in IR.

2.6.2 TNF-α

The *TNF-α* gene is located on chromosome 6p21[176,177] in the class III region of the major histocompatibility complex[177]. Two SNPs, a G-to-A substitution at position -308 (rs1800629), and a C-to-A substitution at position -863 (rs1800630), have been researched and associated with a number of health outcomes, including advanced-stage endometriosis[177] and rheumatoid arthritis[178]. The rs1800629 SNP is the most commonly researched *TNF-α* gene SNP and the A allele has been associated with increased body fat content[179]. It has also been suggested that rs1800629 influences circulating TNF-α levels[32], although this association has not been observed in all studies[180]. In the context of obesity, rs1800630 is understudied. Only two studies have investigated the association of this SNP with obesity, and one found a significant association[176], while the other did not[28]. An association between rs1800630 and circulating TNF-α levels has also been found[181].

Evidence suggests that SNPs in the *TNF-α* gene can modify an individual's risk for obesity-associated complications such as IR[164]. Indeed, these SNPs have been investigated within the context of type 2 diabetes and features of the metabolic syndrome, with mixed results[176]. The results of two European studies of healthy overweight/obese adults did not show an association between IR and rs1800629[180,182]. A study among Mexican women with gestational

diabetes found that participants with the GG genotype had higher HOMA-IR compared to those carrying an A allele[183]. Only one study investigated the association between rs1800630 and IR, with non-significant findings[176]. This highlights the need for more research on this particular variant.

There are a lack of studies on the interaction between *TNF-α* gene SNPs and weight change and its effect on IR. It is important to investigate these relationships in order to determine the best possible intervention to reduce the risk of IR in those most susceptible.

2.6.3 Adiponectin

The gene that encodes adiponectin (*ADIPOQ*) is located on chromosome 3q27 and consists of three exons and two introns[184]. The gene is located in a region where a susceptibility locus for type 2 diabetes and cardiovascular disease has been mapped[184,185]. There are several *ADIPOQ* SNPs that have been identified, with the most common being the rs1501299 (+276 G>T), which is a G-to-T substitution in intron 2 and rs266729 (-11377 C>G), a C-to-G substitution in the immediate 5' flanking region[186]. *ADIPOQ* has been associated with circulating levels of adiponectin, as well as with obesity[187] and obesity-associated co-morbidities[164].

For rs1501299, several studies have found a significant difference between genotypes in circulating adiponectin levels, with individuals of the GG genotype having lower baseline adiponectin compared to T allele carriers[184,188,189]. In contrast, several studies found no such association[190-197]. For rs266729, some studies have found a significant difference between genotypes in adiponectin levels, with individuals of the GG genotype having lower levels compared to C allele carriers[193,198]; other studies have not found such an association[191,192,199].

As with circulating adiponectin levels, the association between *ADIPOQ* SNPs and IR has been equivocal. For rs1501299, studies have revealed a significant association between genotype and HOMA-IR[184,190]; however, one study showed that individuals with the GG genotype had higher baseline HOMA-IR compared to T allele carriers[184], while the other study showed that the TT genotype had higher HOMA-IR compared to G allele carriers[190]. Reasons for the differences could be due to minor allele frequency (MAF) distribution in populations; however, the rs1501299 MAF is similar across all racial/ethnic groups. It is likely that differences in populations and sample sizes are responsible for the disparate results. Fewer studies have examined the role of rs266729 in IR, of which the majority of studies did not find an association between genotype and HOMA-IR. Only one study found a significant difference among genotypes in relation to HOMA-IR[200]. This study was conducted among overweight/obese non-diabetic children (mean age ~11 years) and found participants with the CC genotype to have lower HOMA-IR compared to G allele carriers.

A trend toward an interaction between the rs1501299 SNP and percent body fat in affecting IR has been observed. Also, a significant association between rs1501299 and IR was observed only in those with a higher percent body fat, with G allele carriers with ≥41% body fat having higher HOMA-IR than TT genotype[190].

2.6.4 IL-10

The *IL-10* gene is composed of five exons, and is located in chromosome 1q31-32[37]. The three most commonly researched SNPs in the promoter region of the gene are the G-to-A substitution at position -1082 (rs1800896), a C-to-T substitution at position -819 (rs1800871),

and a C-to-A substitution at position -592 (rs1800872)[201]. These SNPs have been associated with a number of diseases, including Crohn's disease[201], cardiovascular disease[36], and type 2 diabetes[37]. Some studies have found a significant association between *IL-10* SNPs and both obesity and circulating IL-10 levels; however, there is variability in results. For example, a study among overweight/obese Caucasian Italian adults with and without type 2 diabetes found a significant association between genotype for rs1800872 and both BMI and circulating IL-10 levels; individuals with the AA genotype had a higher BMI and lower IL-10 levels compared to C allele carriers[34]. Within the same study, there were no significant differences between genotypes for the rs1800896 SNP and BMI or IL-10 levels. Another study conducted among obese Caucasian Spanish adults found no significant association between genotype for rs1800871 and BMI. However, after morbidly obese participants were removed from the analysis, the association was significant; compared with individuals with the CC genotype, those carrying the T allele had a higher BMI. There was no association between genotype and IL-10 levels in either analyses[35].

Although IL-10 has been shown to be associated with obesity, there is a lack of literature investigating the interrelationships between *IL-10* polymorphisms, obesity, and IR. Only two studies have examined the association between *IL-10* polymorphisms and IR. A study among women with PCOS and healthy controls did not find a difference among the rs1800896 and rs1800871 genotypes for HOMA-IR[202]. The study conducted among overweight/obese Caucasian Italian adults with and without type 2 diabetes also did not find a difference among the rs1800896 genotypes and HOMA-IR, but did find that individuals with the AA genotype for rs1800872 had higher HOMA-IR compared with C allele carriers[34].

2.7 WEIGHT LOSS INTERVENTIONS, CYTOKINE POLYMORPHISMS, AND INSULIN RESISTANCE

How genotype affects IR in response to a dietary, physical activity, and/or behavioral-based weight loss intervention is largely underexplored in the literature. A study among non-diabetic overweight/obese Korean adults enrolled in a 12-week low-calorie dietary intervention found that, for the *ADIPOQ* SNP rs266729, regardless of genotype, individuals were able to significantly reduce HOMA-IR post-intervention. On the other hand, for the adiponectin rs1501299 SNP, individuals with the GG genotype significantly decreased HOMA-IR after the intervention, but T allele carriers did not have a significant decrease in HOMA-IR after weight loss[191]. In a separate study that included Korean adults with impaired fasting glucose or type 2 diabetes who were enrolled in a 12-week low-calorie dietary intervention, HOMA-IR was also significantly reduced post-intervention in participants who had the GG genotype for the rs1501299 SNP; however, this was attenuated when controlling for baseline HOMA-IR levels[192].

Genotypic differences in metabolic outcomes, such as fasting glucose and insulin, resulting from lifestyle modification has been shown. A study among Brazilian adults with pre-diabetes or impaired glucose tolerance investigated the association between genotype and fasting insulin and glucose, after a 9-month lifestyle intervention. For the *TNF-α* SNP rs1800629, individuals with the GG genotype had a significant decrease in insulin post-intervention, whereas those carrying the variant A allele were not able to significantly reduce insulin levels post-intervention. The opposite was true for glucose. For the *IL-6* SNP rs1800795, both individuals with the GG genotype and carriers of the variant C allele were able to significantly reduce insulin post-intervention. Only individuals with the GG genotype were able to reduce glucose

concentration post-intervention[40]. Although this study did not measure IR, the impact of lifestyle modification on the relationship between genotype and other metabolic variables closely related to IR can give clues about how an individual can respond to a lifestyle intervention according to his or her genotype.

2.8 SUMMARY

Obesity rates have reached epidemic proportions over the past few decades, leading to co-morbid conditions such as cardiovascular disease and type 2 diabetes. SBT continues to be among the most effective means of obesity treatment for a wide range of individuals. However, weight maintenance after intentional weight loss is difficult to achieve, and weight regain is common. An individual can then enter a pattern of weight cycling, which can lead to a decline in overall health. Therefore, it is important that more strategies be developed to prevent weight regain and thus improve or maintain health.

IR plays a large role in the development of type 2 diabetes. One of the mechanisms that contribute to IR is dysfunctional adipose tissue, which is a characteristic of obesity. Another mechanism that links obesity to IR is chronic inflammation, where obese states are characterized by an increase in pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines. SBT leading to moderate weight loss can improve both IR and inflammation, although results have not been consistent. Moreover, studies investigating these associations have included

interventions that were short in duration, or collected data at only one time point, limiting the ability to examine the influence of weight regain on these outcomes.

Cytokine gene polymorphisms have been associated with IR, although the results have been inconsistent. Nevertheless, cytokine gene polymorphisms can be informative in determining risk for IR and how an individual responds to a weight loss intervention. Although a few studies have investigated how weight modifies the association between gene polymorphisms and IR, much research is still needed to reduce the ambiguity of this relationship.

Table 2-1. Weight loss studies examining the role of diet and exercise on insulin resistance among overweight/obese participants

Author (Year)	Population	Treatment	Treatment Length	Weight Change	Findings
Naslund et al. (2000)[91]	Obese men (BMI=37.1 kg/m^2, age=42.7 y), non-diabetic N=44	Group sessions (once/wk for 1 h) 1600 kcal/d Moderate exercise encouraged	2 y; FU at 1 and 2 y	baseline-1 y: -5.5% baseline-2 y: -4.1% amount of weight regain 1-2 y: 1.5%	HOMA: Ps had a 14.9% ↓ baseline-1 y ($p<.05$) and a 18.9% ↓ baseline-2 y ($p<.05$); those who gained weight at 2 y had a smaller ↓ in HOMA compared to those who lost at 2 y (-21.6% vs. -15.1%, ns)
Krebs et al. (2002)[72]	Obese women (BMI=1.7 kg/m^2, age=43.7 y), non-diabetic N=57	8-wk milk-based diet (800-900 kcal); food gradually resumed wks 9-12 Groups sessions including info and advice on healthy lifestyle and weight control	12-wk weight loss program + FU at wks 24 and 52 *at 1 yr,	-11.6% baseline-12 wks; -10.6% baseline-24 wks -5.9% baseline-52 wks regain: 12-52 wks: 6.3%	After 12 wks, significant improvement in HOMA (-18.1%, $p<.0001$); no improvements in insulin sensitivity from baseline-52 wks
Villareal et al. (2006)[92]	Obese adults (baseline BMI=39 kg/m^2, age ~71 y), non-diabetic N=27	Treatment: Diet and exercise therapy (n=17) • energy deficit of 750 kcal/d • 90-min exercise session 3 d/wk • group behavioral therapy Control: no treatment (n=10)	26 wks	Treatment group: -8.4%, baseline-26 wks Control group: -0.7% baseline-26 wks	Compared to control group, treatment group had a greater decrease in HOMA post-treatment (-32% vs. 25%, $p<.05$)

Table 2-1 continued

Author (Year)	Population	Treatment	Treatment Length	Weight Change	Findings
Vogeser et al. (2007)[93]	Obese adults (BMI=35.7 kg/m^2, mean age=45 y), Caucasian, non-diabetic N=33	Standard diet (fat limit: 60 g; fruits/vegetables 5 times/d; 3 meals/d) and structured physical exercise on most days	1 y	Median weight loss: -9.0 kg	HOMA ↓ by 45% baseline-1 y ($p<.05$)
Dale et al. (2009)[94]	Obese (BMI≥30 kg/m^2) Caucasian adults with insulin resistance N=72	Treatment: diet and exercise advice Control: continued normal diet and exercise routine	4 months; FU at 24 months	Treatment: -5.5% baseline-4 mos and -1.1% baseline-24 mos Control: -0.4% baseline-4 mos and -0.8% baseline-24 mos Treatment: 4.6% regain 4-24 mos Control: no weight regain 4-24 mos	IS: at 4 mos, treatment group had a greater ↑ in IS compared to control group (11% vs. 8%); at 24 mos, treatment group lower change in IS compared to control group (0% vs. 2%, ns)

Table 2-1 continued

Author (Year)	Population	Treatment	Treatment Length	Weight Change	Findings
Lien et al. (2009)[61]	Obese adults (BMI=32.6 kg/m^2, 18+ y), non-diabetic N=27	Behavioral intervention • Reduced energy intake • Increased exercise • 20 weekly group sessions Those who lost ≥4 kg at initial weight loss randomized to: Group 1: personal phone counseling Group 2: interactive Web site Group 3: Control group	6-mo weight loss period, followed by 6-mo weight maintenance	At 6 mos: -6.3% At 12 mos: -2.8% Weight regain from 6-12 mos: 2.5%	HOMA ↓ by 19.2% baseline-6 mos (p=.01), and by 18.2% baseline-12 mos (p=.0003) % ↑ in HOMA 6-12 mos=1.3%
Goodpaster et al. (2010)[53]	Severely obese adults (BMI 35 kg/m^2 or greater, ages 30-55 y); no major/uncontrolled illnesses N=130	Lifestyle intervention Group 1: diet + initial exercise Group 2: diet + delayed (by 6 mos) exercise Both groups: behavioral lifestyle intervention	1 yr intervention (6- and 12-mo FU)	Baseline-6 mos (p=.02): Group 1: -9% Group 2: -7% Baseline-12 mos (ns): Group 1: -10% Group 2: -8% No regain at 12 mos	No significant group differences in HOMA

Table 2-1 continued

Author (Year)	Population	Treatment	Treatment Length	Weight Change	Findings
Drapeau et al. (2011)[95]	Overweight/obese, postmenopausal women (BMI ~32 kg/m^2, age ~58 yrs), non-diabetic N=46	Calorie restriction (CR) group: 500-800 kcal/d reduction (~1100-1800 kcal/d) CR + resistant training (CR+RT) group: calorie restriction + resistance training (3 d/wk)	6 mos	Mean weight loss at 6 mos: CR: 7.7% CR+RT: 8.2%	HOMA decreased at 6 mos in both groups; however, there was a greater decrease in the CR+RT group compared to the CR group (-12.2% vs. -4.2%; ns)
Mason et al. (2011)[96]	Overweight or obese (BMI≥25.0, ≥23.0 if Asian American), postmenopausal women (50-75 yrs), non-diabetic N=438	Group 1: Dietary weight loss Group 2: Moderate-vigorous exercise Group 3: Diet + exercise Group 4: Control Diet: 1200–2000 kcal/d, 30% kcal fat, 10% weight loss by 6 mos Exercise: 45 minutes, target heart rate of 70%–85% 5 d/wk, by 7th wk Group sessions, meetings with dietitian	6-mo weight loss period, followed by 6-mo weight maintenance	<u>At 12 mos</u> Group 1: -8.5% (p<.0001) Group 2: -2.4% (p=.03) Group 3: -10.8% (p<.0001) Group 4: -0.8%	HOMA at 12 mos by group: Group 1: -24% (p<.0001) Group 2: -9% (p=.19) Group 3: -26% (p<.0001) Group 4: -2% (ref) Participants who lost the most weight had the largest decrease in HOMA at 12 mos: <5% weight loss: HOMA ↓ by 0.2 ≥5-10% weight loss: HOMA ↓ by 0.7 ≥10% weight loss: HOMA ↓ by 1.1 (p<.0001)
Ockene et al. (2012)[97]	Latino/Hispanic adults (BMI ~34 kg/m^2, age=52 y), non-diabetic N=312	Intervention: • individual and group sessions (~60 min) • dietary improvement • increase walking by 4000 steps/d	1 y	Intervention: lost median 2.5 lb at 1 y Usual care: gained median 0.6 lb at 1 y	HOMA significantly improved in the intervention vs. usual care group (median: -0.36 vs. -0.06, p=.03)

Table 2-1 continued

Author (Year)	Population	Treatment	Treatment Length	Weight Change	Findings
Oberhauser et al. (2012)[98]	Obese adults (mean baseline BMI=41.5 kg/m^2, age=42.3 y), non-diabetic, Caucasian N=10	VLCD diet (~800 kcal/d, 4-5 meals/d), nutritional education, behavioral advice, 45 min exercise/wk	3 months	16.5% ↓ in weight post-intervention	HOMA significantly decreased from baseline to end of study (~4.5 to ~2.0, p<.01)
Beavers et al. (2012)[99]	Overweight/obese postmenopausal women (BMI 25-40 kg/m^2, 50-70 y) N=80	Calorie deficit of ~400 kcal/day One counseling session at end of intervention period	Weight loss intervention (baseline-5 mos); post-intervention FU for 12 mos (total=17 mos)	Weight loss during 5-mo intervention: 11.4 kg; 31.4% regain at 17 mos	HOMA ↓ by 38.7% baseline-5 mos, by 18.3% baseline-11 mos, and by 10.6% baseline-17 mos (p<.05) Changes in weight sig. associated with changes in HOMA [b(se)=2.1(0.4), p<.01] Weight regainers had an ↑ in HOMA baseline-12 mos post-intervention, while maintainers had a ↓ in HOMA baseline-12 mos post-intervention (p<.01)
Trussardi Fayh et al. (2013)[100]	Obese adults (BMI 30-39.9 kg/m^2, 22-41 y), non-diabetic N=35	Group 1: Dietary counseling for weight reduction (500-1000 kcal/d reduction) Group 2: Dietary counseling + exercise	Until participants lost 5% of their initial weight	Group 1: -4.5% post-intervention Group 2: -4.8%	Group 1: 19.5% ↓ in HOMA (p<.01) Group 2: 3.2% ↑ in HOMA (ns)

DEXA, dual-energy x-ray absorptiometry; NGT, normal glucose tolerance; IFG, IGT, impaired glucose tolerance; FU, follow-up; HOMA, Homeostatic Model Assessment; IS, insulin sensitivity; VLCD, very low-calorie diet; T2D, type 2 diabetes; ns, non-significant; Ps, participants

Table 2-2 Description of pro- and anti-inflammatory cytokines associated with obesity and insulin resistance

Cytokine	Type/Description	Source	Health Effects Related to Obesity and the Insulin Resistance	References
IL-6	Pro-inflammatory, has some anti-inflammatory properties	Several cells, including endothelial, smooth muscle, adipocytes, monocytes, and fibroblasts	• Reduce glucose uptake • Associated with obesity • Increases TNF-α secretion	103,107,110,203
TNF-α	Pro-inflammatory	Mainly monocytes and macrophages; also includes adipocytes, macrophages, NK cells, and T cells	• Associated with higher BMI and hyperinsulinemia • Inhibits adipogenesis • Increases lipolysis • Impairs insulin signaling	103,131,132
Adiponectin	Anti-inflammatory; three forms (low-, medium-, and high-molecular weight)	Exclusively produced by adipose tissue	• Negative correlation with adiposity • Insulin-sensitizing effects • Low levels associated with type 2 diabetes • Can inhibit TNF-α and IL-6 and enhance production of anti-inflammatory cytokines	131,203,204
IL-10	Anti-inflammatory	M2 macrophages and Th2 lymphocytes	• Inhibits production of pro-inflammatory markers such as TNF-α and IL-6 • Prevents atherosclerotic lesion development and stability • Shown to be both increased and decreased in obesity • May reduce IR through reduction in pro-inflammatory cytokines	89,145-149,152

Table 2-3 Weight loss studies examining the role of diet and exercise on cytokines among overweight/obese individuals

Author (Year)	Population	Cytokine Studied	Treatment	Treatment Length	Weight Change	Findings
Esposito et al. (2003b)[139]	Obese, premenopausal women (BMI 30-49 kg/m^2, 20-46 y), non-diabetic N=120	Adiponectin and IL-6	Intervention group: detailed advice on achieving reduction in weight of ≥10%; education on calorie reduction, goal setting, self-monitoring (3-d food diary) • 1300 kcal/d for 1styr; 1500 kcal/d for 2ndyr Control group: general oral/written information about healthy food choices and exercise	2 y	At 2 y: Intervention: -14.7% Control: -3.2%	Mean % increase in adiponectin, baseline-2 y: • Intervention: 48.2% (p=.02) • Control: 9.3% (p=.13) Mean % decrease in IL-6, baseline-2 y: • Intervention: -32.6% (p=.01) • Control: -7.3% (p=.15)
Monzillo et al. (2003)[130]	Obese (baseline BMI=36.7 kg/m^2), adults (baseline age=49.3 y), various glucose tolerances (8 NGT, 8 IGT, 8 T2D) N=24	Adiponectin, TNF-α, IL-6	Caloric restriction (low calorie, -500 kcal/d) and structured exercise (150 min/wk at 60-80% of participant's max heart rate)	26 wks	-7.0% (all glucose tolerance groups had a similar decrease in weight)	4.3% increase in adiponectin (p=0.76), 17.4% ↓ in TNF-α (p=0.06), and a 14.8% ↓ in IL-6 (p=0.01) after weight loss program; TNF-α sig. ↓ in the IGT group (-28.6%, p=0.02) and adiponectin sig. ↑ in the T2D group (28.8%, p=0.01

Table 2-3 continued

Author (Year)	Population	Cytokine Studied	Treatment	Treatment Length	Weight Change	Findings
Ryan et al. (2003)[154]	Overweight/obese postmenopausal women (BMI=32 kg/m^2, age =57 y), non-diabetic N=40	Adiponectin	Weight loss only (WL): • Calorie restriction (-250-350 kcal/d) • Weekly weight loss classes WL+exercise (WL+EX): • Calorie restriction (-250-350 kcal/d) • Weekly weight loss classes • Supervised exercise 3 times/wk WL+resistance training (WL+RT): • Calorie restriction (-250-350 kcal/d) • Weekly weight loss classes • Supervised resistance exercise 3 times/wk	6 mos	-6.5%	The total sample had a 5.8% ↑ in adiponectin at 6 mos (ns)
Garaulet et al. (2004)[141]	Obese women (baseline BMI=34 kg/m^2, age=37 y), non-diabetic N=33	Adiponectin	Liquid diet (~803 kcal/d)	4 wks	Post-intervention: -7.4%	Non-significant 4% ↓ in adiponectin

Table 2-3 continued

Author (Year)	Population	Cytokine Studied	Treatment	Treatment Length	Weight Change	Findings
Manigrasso et al. (2005)[149]	Android obese (BMI=39 kg/m^2, age=47.2 y), and women, non-diabetic N=15	Adiponectin and IL-10	Weight loss program (20-25% fat, 1200 kcal/d); women encouraged to increase physical activity	12 wks	Median 8% decrease in BMI; 15.9% decrease in weight in 12 women	No significant change in adiponectin (1.0%, ns) or IL-10 (7.3%, ns)
Villareal et al. (2006)[92]	Obese (baseline BMI=39 kg/m^2), adults (baseline age ~71 y), non-diabetic N=27	IL-6	Control: usual diet and exercise routine Treatment: 750 kcal/d deficit, ~30% kcal from fat, goal of 10% weight loss; behavioral therapy	26 wks	Treatment group: -8.2 kg Control group: 0.7 kg	Compared to control group, treatment group had a greater decrease in IL-6 post-treatment (-30% vs. 48%, p<.001)
Belza et al. (2009)[140]	Obese adults (BMI=34 kg/m^2, age 24-62 y), non-diabetic N=33	IL-6	Controlled intervention (all participants): Low-energy diet (LED-1): ~812 kcal/d (1st 8 wks) M-1: weight maintenance (4 wks) LED-2: ~1003 kcal/d (4 wks) M-2: weight maintenance (4 wks) Nutrition education	20 wks	After 20 wks: -14.2%	Compared to baseline, IL-6 significantly reduced at end of study (-21%, p=.02)

Table 2-3 continued

Author (Year)	Population	Cytokine Studied	Treatment	Treatment Length	Weight Change	Findings
Lien et al. (2009)[61]	Adults 18 or older, BMI 30-50 kg/m2, w/o serious medical illnesses N=27	Adiponectin, IL-6, IL-10, TNF-α	Behavioral intervention • Reduced energy intake • Increased exercise • 20 weekly group sessions Those who lost ≥4 kg at initial weight loss randomized to: Group 1: personal phone counseling Group 2: interactive Web site Group 3: Control group	6-mo weight loss period, followed by 6-mo weight maintenance	At 6 mos: -6.3% At 12 mos: -2.8% Weight regain from 6-12 mos: 2.5%	Adiponectin: 0.6% ↑ at 6 mos (ns); levels continued to ↑ at 12 (p=.03) IL-6: 21.9% ↓ at 6 mos (ns); slight ↑ with weight regain at 12 mos (ns) IL-10: no significant changes over time TNF-α: no significant changes over time
Ata et al. (2010)[155]	Overweight/obese, adult premenopausal women (mean baseline BMI ~30 kg/m^2,age 20-45 y), non-diabetic N=70	Adiponectin	Weight loss intervention consisting of 30% protein, 30% fat, and 40% carbohydrate and exercise using a pedometer, increasing steps by 1500 per week	10 wks	Post-intervention: -4.5%	Adiponectin ↑ by 9.6% post-intervention (p<.05)

Table 2-3 continued

Author (Year)	Population	Cytokine Studied	Treatment	Treatment Length	Weight Change	Findings
Christiansen et al. (2010)[138]	Obese adults (BMI 30-<40 kg/m^2, age 18-45 y), Caucasian, non-diabetic N=79	Adiponectin	Exercise only (EX): supervised exercise, 3 times/wk, 60-75 min Hypocaloric diet (DI): liquid very-low calorie diet (600 kcal/d) EX + DI: very-low calorie diet (800 kcal/d) + supervised exercise, 3 times/wk, 60-75 min	12 wks	Post-intervention: EX: -3.5% DI: -11% EX + DI: -11%	Adiponectin post-intervention: EX: ↓ by 6% (ns) DI: ↑ by 19% (p<.01) EX + DI: ↑ by 20% (p<.01)
Fisher et al. (2011)[129]	Overweight premenopausal women (BMI=28 kg/m^2, age 20-41 y), non-diabetic N=126	IL-6 and TNF-α	Diet (D): 800 kcal/d Diet + exercise (D+E): 800 kcal/d + 3 supervised sessions, ~50 min Diet + resistance training (D+R): 800 kcal/d + 3 sessions/wk of 10 exercises related to resistance training	4 wks	Total weight loss: -15.4% By group: D: -15.4% D+E: -15.6% D+R: -15.4%	Total reduction in cytokines: IL-6: -26% (p=.001) TNF-α: -13.2% (p=.049) By group: D: IL-6 (-25.7%), TNF-α (-29.6%), p=.001 D+E: IL-6 (-24.2%), TNF-α (-15.3%), p=.001 D+R: IL-6 (-27.6%), TNF-α (-1.0%) IL-6 time effect: p=.001 TNF-α time effect: p=.001

Table 2-3 continued

Author (Year)	Population	Cytokine Studied	Treatment	Treatment Length	Weight Change	Findings
Lang et al. (2011)[131]	Obese (BMI=30 kg/m^2, waist=100 cm for women, 107 cm for men) adults (age=40 y N=14	Adiponectin and TNF-α	Weight control program 50 min exercise every Saturday Standard low-fat, low-calorie diet (3-day diet diary)	8 wk	-3.2%	Both adiponectin (ns) and TNF-α (ns) were higher after the intervention. No significant association between amount of weight loss and adiponectin or TNF-α
Lira et al. (2011)[156]	Obese adolescents (baseline BMI= ~35 kg/m^2, age=15 y), non-diabetic N=18	Adiponectin, IL-6, IL-10, TNF-α	Dietary and exercise intervention • Dietetics lessons, nutritional consultation • 60-min supervised sessions 3 times/wk	1 yr	Post-intervention: -11%	Adiponectin ↑ by 33% post-intervention ($p<.001$); ↑ in IL-10 and a ↓ in IL-6 and TNF-α, all ns
Rolland et al. (2011)[157]	Obese (BMI≥35 kg/m^2) adults (18 and older), non-diabetic N=31	Adiponectin, IL-6, TNF-α	Low-calorie high-protein diet (LCHP): • 20% kcal carbohydrate, 40% kcal protein, 40% kcal fat • Energy intake: 800-1500 kcal/d Very low-calorie diet (LL): • Meal replacements (soups, shakes, bars) • 550 kcal/d • 36% carbohydrate, 36% protein, 28% fat • Group meetings (behavior change therapy)	9 months	At 9 mos: LCHP = -1.4% LL = -23.8%	From baseline to 9 mos: Adiponectin ↑ by 77.8% in the LL group ($p<.05$) and ↓ by 3.2% in the LCHP group (ns) IL-6 remained the same in the LL group (baseline value=4.4 pg/mL) and ↓ by 13.8% in the LCHP group (from 2.9 to 2.5 pg/mL; ns) TNF-α ↑ by 4.7% in the LL group (ns), and by 2.0% in the LCHP group (ns)

Table 2-3 continued

Author (Year)	Population	Cytokine Studied	Treatment	Treatment Length	Weight Change	Findings
Imayama et al. (2012)[158]	Overweight/obese, postmenopausal women (baseline BMI ~31 kg/m^2, mean baseline age~58 y), non-diabetic N=438	IL-6	Calorie restriction (CR): 1200-2000 kcal/d, depending on weight, ≤30% kcal from fat; individual sessions with dietitian, group sessions Exercise (EX): moderate-to-vigorous, 45 min/d, 5 d/wk CR+EX: both interventions Control: participants' typical diet and exercise routine	1 yr	Weight loss at 6 mos: CR: 6.6 kg EX: 1.0 kg CR+EX: 7.5 kg Control: 0.1 kg	At 1 yr: Compared to control group, IL-6 decreased by 4.5% for EX (p=.49), by 23.1% for CR (p=.001), and by 24.3% for CR+EX (p<.0001)
Tam et al. (2012)[159]	Overweight adults (BMI= 27.8 kg/m^2, age 25-<50 y), non-diabetic N=35	Adiponectin, IL-6, TNF-α	Calorie restriction (CR): 25% CR from baseline energy requirements CR + exercise (CR+EX): 12.5% CR and 12.5% increase in exercise energy expenditure Control: healthy weight maintenance diet	6 mos	CR: -10% CR+EX: -10% Control: -1%	CR: • 14% ↑ in adiponectin • 64% ↑ in TNF-α • 44% ↑ in IL-6 CR+EX: • 7% ↑ in adiponectin • 79% ↑ in TNF-α • 5% ↑ in IL-6 Control: • 6% ↑ in adiponectin • 44% ↑ in TNF-α • 9% ↑ in IL-6 All results ns

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; T2D, type 2 diabetes; ns, non-significant p-value; HRT, hormone replacement therapy

Table 2-4 Description of cytokine gene polymorphisms and obesity-associated health outcomes

Gene (Chromosome Location)	SNP	Polymorphim Location and Position	Minor Allele	Major Allele	Minor Allele Associated with*:	References
Adiponectin (3q27)	rs1501299	Intron 2 at +276	T	G	• Reduced risk for CHD • Higher HDL levels • Lower triglyceride levels • Lower fasting insulin levels • Lower HOMA • Higher plasma adiponectin	190,191,205-207
	rs266729	Promoter -11377	G	C	• Both higher and lower plasma adiponectin • Higher HOMA-IR	191,193,200
TNF-α	rs1800629	-308	A	G	• Higher fasting glucose levels • Increased type 2 diabetes risk • Higher risk of acute coronary syndrome • Increased TNF-α concentration	30,40,208,209
	rs1800630	Promoter -863	A	C	• Lower risk for type 2 diabetes • Lower BMI and waist circumference • Higher TNF-α	176,181
IL-6	rs1800795	Promoter -174	C	G	• Lower fasting glucose independent of BMI • Higher HOMA-IR • Increased IL-6 and BMI	26,163,169,170

Table 2-4 continued

Gene (Chromosome Location)	SNP	Polymorphim Location and Position	Minor Allele	Major Allele	Minor Allele Associated with*:	References
IL-10	rs1800896	-1082	C	T	• Higher risk of acute coronary syndrome • Lower IL-10	201,208
	rs1800871	-819	A	G	• Increased IL-10 • Increased BMI in overweight/obese individuals	35,201
	rs1800872	-592 (C/A)	T	G	• Lower risk of acute coronary syndrome • Lower IL-10 • Higher HOMA levels • Higher fasting insulin levels • Higher BMI	34,208

*compared to major allele carriers
CHD, coronary heart disease

3.0 LONGITUDINAL EFFECTS OF WEIGHT LOSS AND REGAIN ON CYTOKINE CONCENTRATION IN OBESE ADULTS

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3.1 ABSTRACT

Objective: To describe patterns of weight loss and regain and their effect on the pro-inflammatory cytokines IL-6 and TNF-α, and anti-inflammatory cytokines adiponectin and IL-10 during a 24-month weight loss trial.

Methods: Participants were obese adults (N=66) who lost and regained ≥ 10 lbs during a 24-month clinical trial of behavioral weight loss treatment. Measurements of cytokines and weight were conducted at baseline, 6, 12, 18, and 24 months. Linear mixed modeling was used to determine percent change in weight and cytokines from baseline.

Results: The sample was predominantly female (80.3%) and White (86.4%), with a mean age of 48.4±7.3 years and mean BMI of 34.5±4.4 kg/m^2. At baseline, men had higher waist circumference, body weight, and energy intake, and lower percent body fat and adiponectin. The largest decrease in weight was observed at 6 months with a mean 11% decrease ($p<.0001$). A significant gender-by-weight change interaction on percent change in adiponectin was observed [b(se)=0.9 (0.2), p=.0003], with men having a larger increase in adiponectin with weight loss compared to women. There was a significant effect of weight gain over time with increases in IL-6 [b (se) = 0.9 (0.3), p=.001].

Conclusion: Overall, weight loss was significantly associated with improvements in adiponectin and IL-6. Those improvements remained at 24 months, following weight regain. The association between weight change and adiponectin was different between genders. Implementing strategies that support sustained weight loss can help prevent a state of chronic systemic inflammation and its associated adverse effects.

3.2 INTRODUCTION

Weight maintenance after intentional weight loss is difficult to achieve [210-212]. Individuals may develop a pattern of repeated weight loss and regain, which can lead to the development of diabetes [213] and cardiovascular disease [77]. However, there are inconsistencies in the literature regarding the effect of the cyclical pattern of weight loss and regain on an individual's health.

Adipose tissue is an active endocrine organ that produces and secretes pro-inflammatory cytokines (e.g., IL-6 and TNF-α,) and anti-inflammatory cytokines (e.g., adiponectin and IL-10). There is evidence that IL-6 and TNF-α are elevated in obese individuals and decrease with weight loss [214,215]. In contrast, adiponectin and IL-10 are diminished in obese individuals and tend to increase with weight loss [150,216]. Thus, obesity could be viewed as a chronic inflammatory state, characterized by a dysregulation in pro- and anti-inflammatory cytokines.

There is limited literature on long-term weight cycling and cytokine expression. The purpose of our study was to describe the effect of weight cycling on multiple cytokines in a subset of participants who lost and regained at least 10 lbs during the 24-month SMART Trial, a behavioral weight loss study. This prospective study examined the association between changes in weight and changes in cytokines from baseline to 6, 12, 18, and 24 months. To the best of our knowledge, this is the first such report.

3.3 SUBJECTS AND METHODS

3.3.1 Study Design

The current study was a secondary analysis of data from the SMART Trial (NCT00277771), a single-centered, 24-month clinical trial of behavioral weight loss treatment that used a repeated measures design. The recruitment, screening, and enrollment details of the SMART Trial are published elsewhere[217]. Briefly, 210 participants were randomized to one of three conditions: 1) use of a standard paper diary; 2) use of an electronic diary with dietary and physical activity self-monitoring software; or 3) an electronic diary plus a customized feedback program for self-monitoring of diet and physical activity. The aim of the study was to determine whether self-monitoring of daily food intake and physical activity using a mobile device, with or without a daily, tailored feedback message, was superior to using a paper diary for short- and long-term weight loss.

3.3.2 Participant Characteristics

A sub-group of 66 SMART Trial (NCT00277771, [217]) participants who lost and regained ≥10 lbs at any time in the 24-month study were included in the analysis. Individuals were included if they were 18-59 years of age and had a BMI between 27 and 43 kg/m^2. Detailed eligibility criteria were published elsewhere [217]. Participants provided written informed consent; the study was approved by the University of Pittsburgh Institutional Review Board.

3.3.3 Intervention

All participants received the standard behavioral intervention, which included: 1) self-monitoring of diet and exercise; 2) group sessions; 3) daily energy and fat intake goals; and 4) weekly exercise goals.

3.3.3.1 Self-monitoring

Participants in the paper diary group were given standard paper diaries and instructed to record all foods eaten and the calorie and fat content (in grams), as well as minutes of exercise. Participants in the other two groups were provided a mobile device that contained dietary self-monitoring software that tracked energy and fat intake and displayed on the screen of the device current intake related to daily goals; the software included a USDA-based, 6 000-item food database [DietMate Pro©[218,219]] and also software that permitted self-monitoring of physical activity (CalcuFit [PICS, Reston, VA]). At each session, paper diary participants submitted their diaries and received new ones to use until the next session. The interventionist reviewed those diaries, provided written feedback and returned the diaries at the next session. Participants in the other two groups turned in their mobile devices at the beginning of the session, the self-monitoring data were uploaded to the study computer and the devices were returned to participants at the end of the session. The interventionists received printed reports that appeared similar to the standard paper diaries for their review. The reports with the interventionists' written comments were returned to the participants at the next group session. In addition to the

feedback received on current energy and fat intake displayed on the screen of the mobile device next to the daily goals, the FB group received a daily message that reminded them to record, or if they had recorded, was responsive to the information they had entered. For example, if an individual was at risk for exceeding their energy goal at lunch but was within limits of their fat goal, a message would be delivered that read “Nice job limiting fats; might want to limit sweets/candy this afternoon.” While both groups received general feedback to the dairy at the next group session, the FB group received a daily message that was tailored to the diary entries made that day and delivered remotely in real time.

3.3.3.2 Group sessions

There were 39 group sessions, occurring weekly in months 1-4, bi-weekly in months 5-12, and once a month in months 13-18. One session held during the final six months of the study focused on weight maintenance. Sessions included instructions in developing healthy eating and physical activity habits, group problem-solving exercises, and use of behavioral change strategies; they also provided practical hands-on experiences to develop skills related to implementing a healthy lifestyle.

3.3.3.3 Dietary and exercise goals

Each participant received a daily energy and fat gram goal based on her/his gender and baseline weight, consistent with standard behavioral weight loss treatment [210]. The daily energy intake goal was between 1 200 and 1 500 calories for females and between 1 500 and 1 800 for males; the fat allowance was 25% of total calories for all participants. Participants were instructed to gradually develop an exercise program and aim to reach a weekly goal of 150 min

of moderate intensity exercise by the 6th week; the goal was increased by 30 minutes every six months.

3.3.4 Measures

A medical history form and sociodemographic data questionnaire were completed at baseline. Energy intake was measured in kilocalories per day (kcal/day) by two unannounced 24-hour dietary recalls, conducted on one leisure day and one work day. A blood sample was obtained for total adiponectin, IL-10, IL-6, and TNF-α at baseline, 6, 12, 18, and 24 months.

3.3.4.1 Anthropometric measurements

Height was measured with a wall-mounted stadiometer and recorded in centimeters. Weight and percent body fat were measured following an overnight fast using a Tanita Scale and Body Fat Analyzer (Tanita Corporation of America, Inc., Arlington Heights, IL) while participants wore light clothing and stood erect with bare feet on the scale's footpads.

3.3.4.2 Cytokine measurements

TNF-α was measured by Luminex technology multiplex ELISA (Linco Research, Inc.; St. Charles, MO). The minimum detectable level (MDL) was 0.14 pg/mL, with an assay range of 0.64-10,000 pg/mL. The intra- and inter-assay CVs were 1.4-7.9% and <21%, respectively. Quantitative sandwich enzyme immunoassay technique was used to measure IL-6 (Quantiglo Human IL-6 Immunoassay; R&D Systems, Minneapolis), with the MDL ranging from 0.05-0.35

pg/mL and an assay range of 0.48-1,500 pg/mL. The intra- and inter-assay CVs were 3.0-5.8% and 6.3-9.6%, respectively.

IL-10 was measured by quantitative sandwich enzyme immunoassay technique (Quantikine HS Human IL-10 Immunoassay; R&D Systems, Minneapolis). The MDL was <0.5 pg/mL, with an assay range of 0-50 pg/mL. The intra- and inter-assay CVs were 6.6-8.5% and 8.1-15.6%, respectively. Finally, the ELISA technique was used for the adiponectin assay (Quantikine Human Adiponectin/Acrp30 Immunoassay; R&D Systems; Minneapolis), with a MDL of 0.25 ng/mL and intra- and inter-assay CVs of 2.5-4.7% and 5.8-6.9%, respectively All cytokine assays were performed at the Laboratory for Clinical Biochemistry Research at the University of Vermont.

3.3.5 Data Analysis

Summary statistics were reported as mean (SD) and frequency count (%). All non-normal weight and cytokine variables were log-transformed. Independent sample t-tests were used to compare baseline means between women and men. Spearman correlations were used to test the associations between baseline body weight and cytokines. Percent change from baseline to 6, 12, 18 and 24 months were used in the analyses of weight and cytokines. Percent change from baseline was calculated as [(follow-up – baseline)/ baseline) x 100]. Linear mixed modeling was applied to assess the effects of weight change since baseline on change in cytokines from baseline, adjusting for time, age, race/ethnicity, gender, baseline body weight, baseline energy intake, baseline cytokine concentration, and percent change in energy intake. To determine whether associations between percent change in weight and percent change in cytokines varied

by gender, an interaction between gender and percent weight change was included in the models. In each cytokine model, a random intercept was used for each participant, and an unstructured covariance structure was assumed. P-values from the F-tests were reported. In all analyses, the Bonferroni approach was used to correct for multiple testing (four tests: adjusted p=.0125). Analyses of the data were conducted using SAS version 9.2 (SAS Institute, Cary, NC) and IBM SPSS Statistics version 21.0 (IBM Corporation, Armonk, NY).

3.4 RESULTS

Baseline characteristics for women and men are displayed in Table 3-1. Participants' mean age was 48.4 years. Compared to women, men had significantly higher weight, waist circumference, energy intake, and adiponectin, but significantly lower percent body fat.

3.4.1 Associations between baseline weight and baseline cytokines

Weight was significantly correlated with adiponectin ($r = -.31$, $p=.01$), IL-6 ($r = .41$, $p=.001$), and TNF-α ($r = .43$, $p<.0001$), but there was no significant correlation between IL-10 and weight ($r = .05$, $p=.68$). There were significant correlations between cytokines, including adiponectin and IL-6 ($r = -.33$, $p=.01$), adiponectin and TNF-α ($r = -.31$, $p=.01$), and IL-6 and TNF-α ($r = .25$, $p=.04$). There were no significant associations between IL-10 and the other cytokines.

3.4.2 Description of percent change in weight and cytokines by time

Description of the percent changes in weight and cytokines over time are displayed in Figure 3-1. The largest decrease in weight was observed at 6 months (-11%, p<.0001), with evidence of regain at 12, 18, and 24 months. Concurrent with baseline to 6-month weight loss, adiponectin increased by 13.4% (p<.0001). From baseline to 12 months, adiponectin significantly increased by 23.8% (p<.0001), while IL-6 significantly decreased from baseline (-18.1%, p=.0003). Baseline to 18-month changes were significant only for adiponectin (19.1%, p=.0001); at 24 months, adiponectin was significantly higher than at baseline (9.9%, p=.004) but was lower than the 18-month values. There were no significant changes in IL-10 or TNF-α at either time points.

3.4.3 The associations between percent change in weight and percent changes in cytokines

Linear mixed models results on the associations between percent change in weight and in cytokines are shown in Table 3-3. Controlling for covariates, a significant interaction between gender and percent change in weight on percent change in adiponectin over time was detected [b(se)=0.9 (0.2), p=.0003], with men having a higher percent increase in adiponectin with weight loss, compared to women with the same amount of weight loss (Figure 3-2). For example, if men had an overall weight loss of 20%, they increased their adiponectin concentration by about 20%; however, women with the same amount of weight loss only increased their adiponectin concentration by about 10%. The opposite was true for weight regain; compared to women, men had greater decreases in adiponectin with the same amount of weight regain. Change in weight

was significantly associated with change in IL-6 [b(se)=0.9 (0.3), p=.001], and marginally associated with change in IL-10 [b(se)=0.3 (0.2), p=.07]. There was no significant association between weight changes and TNF-α changes [b(se)=-0.1 (0.1), p=.22].

3.5 DISCUSSION

We observed improvements in cytokines after weight loss, and despite participants regaining most of their lost weight by 24 months, improvements in cytokines persisted. Moreover, we demonstrated that decreases in weight predicted an increase in adiponectin and a decrease in IL-6. We also showed that the association between weight change and change in adiponectin was different for men and women. Although there were significant associations between weight and adiponectin, IL-6, TNF-α at baseline, only adiponectin was significantly associated with weight loss, and an 11% weight loss led to a significant increase in adiponectin. Because adiponectin is exclusively released from adipose tissue, it may be more sensitive to the hypertrophic changes in adipocytes. Conversely, IL-6, TNF-α, and IL-10 originate from a variety of sources, which can affect serum expression of cytokines during weight changes. Although the weight-loss associated increases in adiponectin have been well-described[143,144,155,220,221], there are inconsistencies regarding changes in the other cytokines with weight loss. Sofer et al. found IL-6 significantly decreased at 6 months of weight loss treatment but did not find any significant changes in TNF-α [222]. In contrast, Pakiz et al. found that TNF-α significantly decreased with weight loss with no significant changes in IL-6 [223]. Methodological differences among these studies may have contributed to the variability of the results.

We observed continued improvements in cytokines from the 6-month point, demonstrating that the improved cytokine concentration occurring with weight loss can be sustained despite weight regain. This suggests a delayed effect of weight regain on cytokine concentration. At 24 months, adiponectin declined and IL-6 increased from the point of greatest weight loss, suggesting that regain attenuates the effects of weight loss on some cytokines. Our findings that improvements in cytokines persist despite weight regain support similar findings reported by others [61,144]. However, Erez et al. found a positive association between adiponectin and weight loss but no significant association between adiponectin and weight regain [143].

Baseline correlation did not reveal significant associations between IL-10 and adiposity measures or other cytokines. There is evidence that IL-10 is reduced in obese states [149]. However, the evidence on IL-10 and weight change is limited, making this association unclear. We noticed that men had greater increases in adiponectin with weight loss than women, despite both groups having the same amount of weight loss. We also noticed that men had greater decreases in adiponectin with regain than women, despite both groups having the same amount of regain. This suggests that weight loss studies should target men, as they may have great health benefits from weight loss.

The only study similar to ours was conducted by Lien et al. who described changes following a 6-month behavioral weight loss intervention followed by weight regain [61]. They did not observe a significant change in adiponectin levels concurrent with the weight loss, nor did they observe any significant changes in IL-6 during weight loss or regain. However, they observed an increase in adiponectin from baseline to post-weight regain, also showing the sustained improvement in adiponectin despite weight regain [61].

Bluher et al. examined cytokines in participants enrolled in a study of Mediterranean diet vs. low carbohydrate diet [144] and demonstrated an increase in adiponectin with weight loss; however, there was a continued increase in adiponectin levels at 24 months. There are several differences between Bluher et al.'s and the current study. First, Bluher et al. included participants with type 2 diabetes, those with varying weight change patterns (e.g. maintained, lost or regained weight), and did not restrict energy intake whereas our study recommended a restricted calorie and 25% fat eating plan. Additionally, women represented 14% of their sample while 80% of our participants were women. Lastly, Bluher et al. used a trend analysis that precluded them from determining whether the association between weight change and change in cytokines was statistically significant [144]. It is possible that failing to control for part of the sample that maintained their weight may have contributed to the observed increase in adiponectin at 24 months in Bluher et al.'s study. Limiting the sample to only participants who lost and regained weight precludes the occurrence of bias that may occur when including individuals who maintained the weight loss.

Regarding study limitations, we did not have a control group with which to compare our results. Although we found significant associations between changes in weight and changes in cytokines, we cannot make any causal inference. We included healthy individuals within specific ages and BMIs, thus our results cannot be generalized to dissimilar populations. Our study also has several strengths. We studied a unique population of individuals who lost and regained ≥10 lbs and also incorporated a longitudinal study design with data collected at five time points over two years. In summary, changes in weight were associated with changes in adiponectin and IL-6. The improvement in these cytokines persisted even with nearly total weight regain, suggesting that weight loss can have protective effects despite weight regain. Because the majority of

individuals who lose weight experience weight regain, it is important to develop strategies to prevent regain to reduce the duration of exposure to an inflammatory state, thus reducing the risk of developing chronic diseases such as diabetes and atherosclerosis.

Table 3-1 Mean (SD) baseline characteristics of the participants

Characteristic	Women (n=53)	Men (n=13)	Total (N=66)
Age (years)	47.6 (7.3)	51.5 (7.2)	48.4 (7.4)
Weight (kg)*	92.8 (13.7)	111.9 (14.3)	96.5 (15.7)
BMI (kg/m^2)	34.3 (4.3)	35.4 (4.6)	34.5 (4.4)
Waist (cm)*	105.1 (12.3)	119.3 (11.4)	107.9 (13.3)
Energy intake (kcal/day)*	2029 (480)	2781 (696)	2177 (604)
Body Fat Composition (%)*	44.0 (4.5)	33.9 (5.3)	42.0 (6.1)
Adiponectin (μg/mL)*	18.2 (9.5)	11.6 (5.2)	16.9 (9.2)
IL-10 (pg/mL)	8.8 (4.3)	8.7 (3.6)	8.8 (4.1)
IL-6 (pg/mL)	2.5 (1.4)	2.8 (1.7)	2.6 (1.5)
TNF-α (pg/mL)	5.0 (4.8)	5.5 (1.7)	5.1 (4.3)

kg=kilograms; kg/m^2=kilograms per square meters; cm=centimeters; kcal/day=kilocalories per day; MET-h/wk=metabolic equivalent of task-hours per week; μg/mL=micrograms per deciliter; pg/mL=picograms per deciliter

*$p<.05$ women vs. men from t-tests

Table 3-2 Description of percent change in weight and cytokines from baseline to 6, 12, 18, and 24 months

Characteristic	6 months	12 months	18 months	24 months
	Mean (SE[1])	Mean (SE[1])	Mean (SE[1])	Mean (SE[1])
Weight	-10.7 (0.6)*	-9.6 (0.9)*	-5.1 (0.9)*	-3.3 (0.8)*
Adiponectin	13.4 (2.9)*	23.8 (4.4)*	19.1 (4.7)*	9.9 (3.3)*
IL-10	2.5 (4.8)	8.8 (5.1)	4.8 (5.3)	12.4 (10.8)
IL-6	-7.7 (4.5)	-18.1 (4.8)*	-4.6 (5.0)	-1.1 (9.2)
TNF-α	-5.6 (4.7)	-4.2 (6.9)	-3.6 (4.9)	0.4 (6.3)

[1]standard error
*significant difference from baseline ($p<.05$)
Results are least squares means

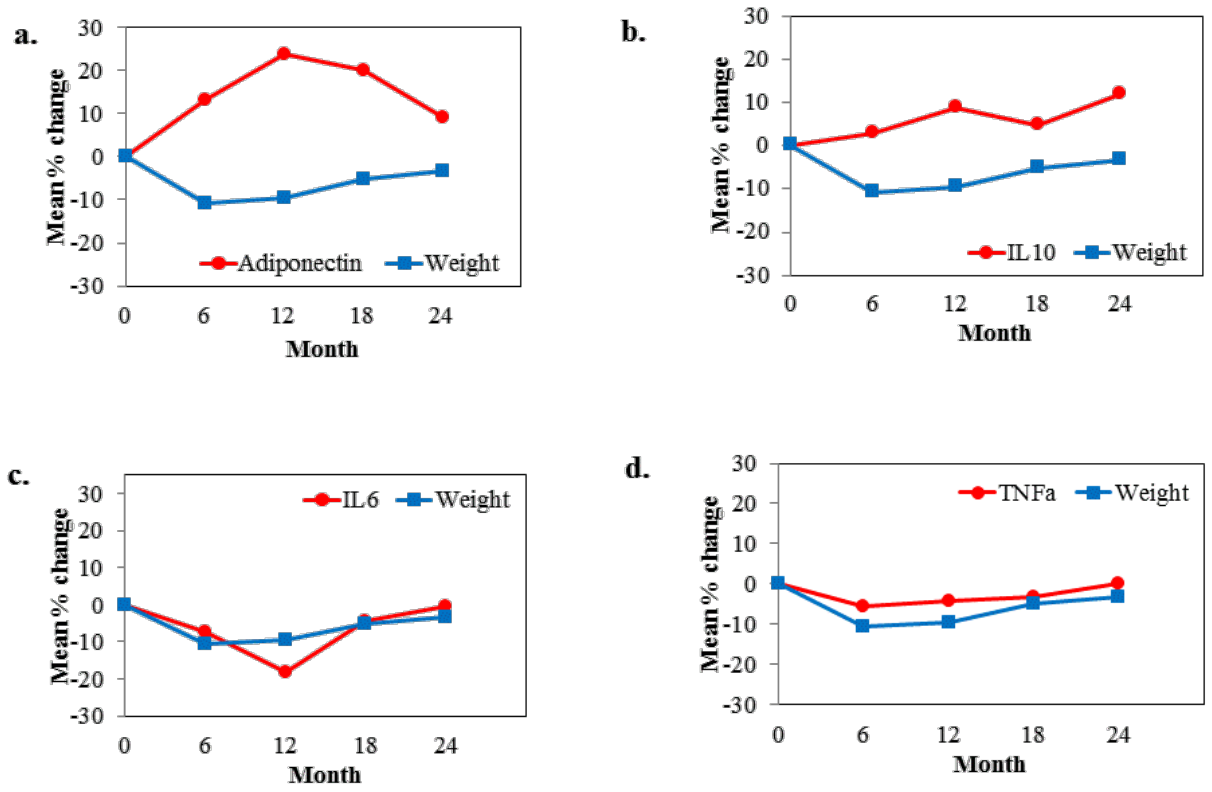

Figure 3-1 Description of percent change in weight and percent change in cytokines over time (N=66)

The red line represents mean percent change in: a. adiponectin; b. IL-10; c. IL-6; and d. TNF-α. The blue line represents mean percent change in weight. Results are least squares means and standard errors from linear mixed models.

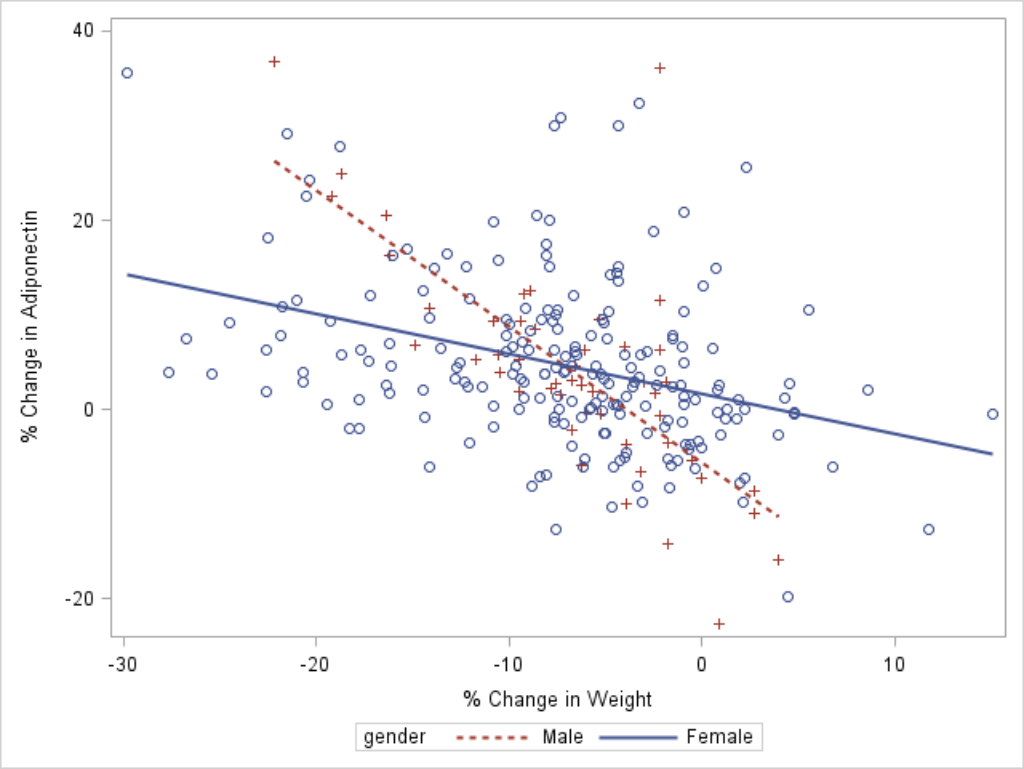

Figure 3-2 Scatterplot of the interaction between gender and percent weight change on percent change in adiponectin over time

Note: The scatterplot represents multiple time points per individual

Table 3-3 Percent change in cytokines over time for every percent change in weight (N=66)[1]

Cytokine[2]	B	SE	P
Adiponectin	-2.2	0.5	<.0001
IL-10	0.3	0.2	.07
IL-6	0.9	0.3	.0001
TNF-α	-0.1	0.1	.22

[1]Models adjusted for time, age, race/ethnicity, gender, baseline weight, baseline energy intake, baseline log-transformed cytokine concentration (either adiponectin, IL-10, IL-6, or TNF-α, depending on the outcome), and percent change in energy intake
[2]Percent change in log-transformed cytokine

4.0 ASSOCIATIONS BETWEEN WEIGHT LOSS AND REGAIN AND METABOLIC MEASURES IN OBESE ADULTS: RESULTS FROM A 24-MONTH BEHAVIORAL WEIGHT LOSS TRIAL

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4.1 ABSTRACT

Background: Obesity is linked to the development of insulin resistance (IR) and type 2 diabetes. Weight loss achieved by lifestyle modification could improve insulin sensitivity. However, weight loss is difficult to maintain, and individuals often weight cycle, potentially placing them at greater risk for adverse health outcomes.

Objective: The purpose of this secondary analysis was to examine how long-term changes in weight are associated with changes in factors related to the development of diabetes (insulin concentration and IR) among obese adults enrolled in a 24-month behavioral weight loss trial.

Methods: The sample (N=66) included non-diabetic adults who lost and regained at least 10 lbs during the 24-month trial. All participants received standard behavioral weight loss treatment during the first 18 months. Assessments were conducted at baseline, 6, 12, 18, and 24 months. IR was assessed using the homeostatic model assessment (HOMA-IR). Linear mixed modeling was used to examine the association between % changes in weight and % changes in fasting insulin, and HOMA-IR. All models were adjusted for time, age, gender, race, baseline weight, and baseline metabolic measure.

Results: The sample was 80% female and 86% White. At baseline, the mean (±SD) values were: age, 48.4±7.4 years; BMI, 34.5±4.4 kg/m^2; plasma insulin, 16.9±6.0 μU/mL; fasting glucose, 96.6±8.8 mg/dL; and HOMA-IR, 4.1±1.7. On average, compared to baseline, participants experienced an 11% decrease in weight ($p<.0001$), a 9% decrease in insulin ($p=.004$), and a 10% decrease in HOMA-IR ($p=.01$) at 6 months. Following 6-month weight loss, an average of 8.4% of baseline weight was regained by 24 months ($p<.0001$), and the positive changes observed in the other measures were partially reversed, e.g., a 19% increase in insulin ($p=.001$), and a 26%

increase in HOMA (p=.001). Overall, weight change was positively associated with changes in insulin [b(se)=0.5(0.1), p≤.0001] and HOMA [b(se)=0.8(0.2), p≤.0001].

Conclusion: In this sample of participants who weight cycled during a 24-month trial, weight loss improved metabolic outcomes; however, this was attenuated when weight was regained. These results reveal the effects of changes in weight on metabolic outcomes and highlight the importance of sustaining healthy lifestyle changes that support weight loss maintenance and improved metabolic outcomes.

4.2 INTRODUCTION

Obesity has become one of the most prevalent health issues. The global prevalence of obesity is approximately 500 million, and by 2015, it is expected to reach approximately 700 million[45]. Currently in the U.S., about 36% of adults are obese[1], and the current obesity epidemic is partly responsible for the increase in diseases such as hypertension, diabetes, and the metabolic syndrome[49]. Insulin resistance (IR), characterized by reduced glucose uptake in targeted peripheral tissues[4], is present in obesity and is considered a mediator in the obesity-type 2 diabetes pathway.

Lifestyle modification, consisting of decreased energy intake, increased energy expenditure, and behavioral modification, is considered the cornerstone to weight loss treatment[6]. Currently, about 57% of women and 40% of men are attempting to lose weight[9], and moderate weight loss of about 5-10% of initial weight is associated with improvements in obesity-associated comorbidities[8]. Often, individuals succeed in short-term weight loss; however,

long-term weight maintenance is difficult to achieve[58], and many individuals who intentionally lose weight often regain all or part of the lost weight within 1-5 years post-weight loss[56,57,60]. These individuals can enter into a pattern of weight loss and regain, or weight cycling, which can have detrimental health effects.

Although modest weight loss can improve health outcomes such as IR, it is unclear whether these improvements remain or are attenuated when weight is regained[61]. Very few studies have addressed the effect of weight regain on IR. Of the studies that have addressed this issue, the results have been mixed. Some studies have found a significant association[75], while other studies have not[76,77]. Many of these studies are limited because of short duration of weight loss intervention, measuring individuals at only one or two time points, cross-sectional rather than longitudinal studies, inclusion of both unintentional and intentional weight loss[78], and lack of detail regarding amount and timing of weight regain[76,78]. Therefore, the purpose of this study is to describe the effect of weight cycling on metabolic variables in a subset of participants who lost and regained at least 10 lbs during the 24-month SMART Trial, a behavioral weight loss study. This prospective study examined the association between changes in weight and changes in insulin, glucose, IR, and insulin sensitivity (IS) across four time periods: from baseline to 6, 12, 18, and 24 months.

4.3 METHODS

4.3.1 Study Design

The current study was a secondary analysis of data from the SMART Trial (NCT00277771), a single-centered, 24-month clinical trial of behavioral weight loss treatment that used a repeated measures design. Details of recruitment, screening, and enrollment are outlined elsewhere[217]. Briefly, 210 individuals were randomized to one of three conditions: 1) use of a standard paper diary; 2) use of an electronic diary with dietary and physical activity self-monitoring software; or 3) an electronic diary plus customized feedback program for self-monitoring of diet and physical activity. The aim of the study was to determine whether self-monitoring of daily food intake and physical activity using a mobile device, with or without a daily, tailored feedback message, was superior to using a paper diary for short- and long-term weight loss.

4.3.2 Participant Characteristics

A total of 66 SMART Trial subjects who lost ≥10 lbs of body weight and later regained ≥ 10 lbs were included in the analysis. The weight loss and regain occurred at any time in the 24-month study. Individuals were eligible for inclusion in the parent study if they were 18-59 years of age with a BMI between 27 and 43 kg/m^2, were willing to be randomized to one of the three treatment conditions, and had completed five-day recording of food intake in a paper diary. Individuals were excluded from the study if they had physical limitations that prohibited their

ability to exercise; reported alcohol intake of ≥4 drinks/day; presented with an eating disorder; participated in a weight loss program or used of weight loss medication within the 6 months prior to enrollment; planned an extended vacation, absence, or relocation during the study, were pregnant or planning to become pregnant during the study; and had a current serious illness or unstable condition requiring physician-supervised diet and exercise, including a glucose level above 125 mg/dL at baseline. All participants provided written informed consent, and the study protocol was approved by the University of Pittsburgh Institutional Review Board.

4.3.3 Intervention

All participants received the standard behavioral intervention, which included: 1) self-monitoring of diet and exercise; 2) group sessions; 3) daily energy and fat intake goals; and 4) weekly exercise goals.

4.3.3.1 Self-monitoring

Participants in the paper diary group were given standard paper diaries and instructed to record all foods eaten and the calorie and fat content (in grams), as well as minutes of exercise. Participants in the other two groups were provided a mobile device that contained dietary self-monitoring software that tracked energy and fat intake and displayed on the screen of the device current intake related to daily goals; the software included a USDA-based, 6000-item food database [DietMate Pro©[218,219]] and also software that permitted self-monitoring of physical activity (CalcuFit [PICS, Reston, VA]). At each session, paper diary participants submitted their diaries and received new ones to use until the next session. The interventionist reviewed those

diaries, provided written feedback and returned the diaries at the next session. Participants in the other two groups turned in their mobile devices at the beginning of the session, the self-monitoring data were uploaded to the study computer and the devices were returned to participants at the end of the session. The interventionists received printed reports that appeared similar to the standard paper diaries for their review. The reports with the interventionists' written comments were returned to the participants at the next group session.

4.3.3.2 Dietary and exercise goals

Each participant received a daily energy and fat gram goal based on her/his gender and baseline weight, consistent with standard behavioral weight loss treatment[210]. The daily energy intake goal was between 1200 and 1500 calories for females and between 1500 and 1800 for males; the fat allowance was 25% of total calories for all participants. Participants were instructed to gradually develop an exercise program and aim to reach a weekly goal of 150 min of moderate intensity exercise by the 6th week; the goal was increased by 30 minutes every six months.

4.3.3.3 Group sessions

There were 39 group sessions, occurring weekly in months 1-4, bi-weekly in months 5-12, and once a month in months 13-18. One session held during the final six months of the study focused on weight maintenance. Sessions included instructions in developing healthy eating and physical activity habits, group problem-solving exercises, and use of behavioral change strategies; they also provided practical hands-on experiences to develop skills related to implementing a healthy lifestyle.

4.3.3.4 Feedback

In addition to the feedback received on current energy and fat intake displayed on the screen of the mobile device next to the daily goals, the FB group received a daily message that reminded them to record, or if they had recorded, was responsive to the information they had entered. For example, if an individual was at risk for exceeding their energy goal at lunch but was within limits of their fat goal, a message would be delivered that read "Nice job limiting fats; might want to limit sweets/candy this afternoon." While both groups received general feedback to the dairy at the next group session, the FB group received a daily message that was tailored to the diary entries made that day and delivered remotely in real time.

4.3.4 Measures

Height was measured with a wall-mounted stadiometer and recorded in centimeters. Weight and percent body fat were measured following an overnight fast using a Tanita Scale and Body Fat Analyzer (Tanita Corporation of America, Inc., Arlington Heights, IL) while participants wore light clothing and stood erect with bare feet on the scale's footpads. Blood samples were obtained for assessment of insulin and glucose levels, and both metabolic measures were analyzed at the University of Pittsburgh Graduate School of Public Health's Chemistry and Nutrition Laboratory. Insulin was measured using a radioimmunoassay procedure developed by Linco Research, Inc. Glucose samples were quantitatively determined by an enzymatic method described by Bondar and Mead (1974)[224]. The coefficient of variation (CV) for insulin was 8.2 +/- 0.7 (170) %) and was 2.0% for glucose. The intra-assay CVs for insulin and glucose were 7.0% and 1.6%, respectively. Insulin resistance was assessed by the Homeostatic Model

Assessment of Insulin Resistance (HOMA-IR), calculated as [fasting insulin (μU/mL) x fasting glucose (mg/dL)] / 405.

4.3.5 Data Analysis

Descriptive statistics were calculated as mean (standard deviation) for continuous variables and proportions for categorical variables. Variables were assessed for normality, and non-normal variables were log-transformed. Simple linear regression was used to determine whether covariates were associated with baseline insulin and HOMA-IR. Covariates included were age, race, gender, and baseline: BMI, weight, waist circumference, metabolic variables, energy intake, adiponectin, IL-6, IL-10, and TNF-α. If covariates had a $p<.10$, they were examined as part of the multivariable regression model. Any covariates that were statistically significant at $p<.10$ from the multivariable model were covariates used for the linear mixed model analyses. Percent change from baseline to 6, 12, 18 and 24 months were used in the analyses of weight and cytokines. Percent change from the study entry time point was calculated as [(follow-up – baseline)/ baseline) x 100]. Linear mixed modeling with linear contrasts was applied to assess the effects of weight change since baseline on changes in insulin and HOMA-IR from baseline. The models were adjusted for age; race; gender; baseline: BMI, weight, insulin or HOMA-IR, and energy intake; and percent change in energy intake. The interaction between gender and percent change in weight was also tested and dropped from the model if not significant. In each metabolic model, a random intercept was used for each participant, and an unstructured covariance structure was assumed. P-values from the F-tests were reported. In all analyses, the significance level was set at $p = 0.05$ for two-sided hypothesis testing. Analyses of

the data were conducted using SAS version 9.2 (SAS Institute, Cary, NC) and IBM SPSS Statistics version 21.0 (IBM Corporation, Armonk, NY).

4.4 RESULTS

Baseline demographic, anthropometric, and clinical characteristics of the participants (N=66) are shown in Table 4-1. The majority of participants were middle-aged (48.4±7.4 years), female (80%), White (86.4%), and educated (16.1±3.2 years). Compared with women, men had significantly higher body weight, waist circumference, insulin, fasting glucose, and HOMA-IR, but significantly lower percent body fat.

4.4.1 Description of percent change in weight and metabolic measures over time

Percent changes in weight and metabolic measures from baseline to 6, 12, 18, and 24 months are outlined in Table 4-2 and Figures 4-1 and 4-2. On average, participants lost approximately 11% of their baseline body weight at 6 months ($p<.0001$), and there was evidence of regain at 12, 18, and 24 months. Concurrent with baseline to 6-month weight loss, there were positive changes in insulin and HOMA-IR. Specifically, there were decreases in insulin (-8.9%, $p=.004$) and HOMA-IR (-9.6%, $p=.01$) from baseline to 6 months. By 24 months, improvements in metabolic measures were not sustained. Specifically, there were 5% and 8% increases in insulin and HOMA-IR, respectively, from baseline to 24 months. However, none of these changes at 24 months were significant.

4.4.2 The associations between percent change in weight and percent changes in metabolic measures

Table 4-3 outlines results of linear mixed models examining the association between percent changes in weight and metabolic measures over time, controlling for time, age, race, gender, and baseline weight and metabolic measures. Change in weight was significantly associated with insulin [b(se)=0.5(0.1), p≤.0001] and HOMA-IR [b(se)=0.8(0.2), p≤.0001]. Specifically, weight loss was associated with a 0.5% decrease in insulin (p≤.0001) and a 0.8% decrease in HOMA-IR (p≤.0001).

4.5 DISCUSSION

In this secondary analysis of a 2-year behavioral weight loss intervention trial of participants who lost and regained ≥10 lbs, we observed decreases in metabolic measures with moderate weight loss. However, with nearly total weight regain at 24 months, these improvements were not sustained. Moreover, we found that decreases in weight predicted decreases in insulin concentration and HOMA-IR. Our findings of improved metabolic measures after moderate intentional weight loss are supported by the literature. A 6-month weight loss study by Antuna-Puente et al. found that, with a 6% decrease in BMI, HOMA-IR and fasting insulin concentration each significantly decreased by 12%[225]. Likewise, a study by Rector et al. found a calorie-restricted and moderate-intensity exercise program that produced a 9% weight loss at 6 months decreased HOMA-IR by 28%[226].

Although there is a general consensus in the literature that weight loss improves metabolic measures, there is inconsistency on the role of weight regain in metabolic outcomes. Very few studies have investigated the effect of weight regain after intentional weight loss on metabolic measures. Lien et al.[61] discovered that, in obese adults who lost 6% of their initial body weight at 6 months of a behavioral weight loss intervention, HOMA-IR and insulin were significantly lower at end of study (12 months) compared to baseline, despite the weight regain. Unlike Lien et al., we did not observe persistent metabolic improvement during weight regain. This may be due to participants in Lien et al.'s study having partial weight regain at 12 months, whereas participants in our study had nearly total regain at 24 months. Also, Lien et al.'s study was one year long; it is possible that if they had followed their participants for 2 years, they may have seen higher insulin concentration and HOMA-IR at conclusion of the study.

When investigating differences in insulin and HOMA-IR from baseline to each time point, we observed a 9% and 10% decrease in insulin and HOMA-IR, respectively, at 6 months, corresponding with the 11% decrease in weight. With weight regain at 24 months, we observed a 5% and 8% increase in insulin and HOMA-IR, respectively. However, results were only significant during the weight loss period. Beavers et al.[99] investigated changes in cardiometabolic outcomes among participants in a weight loss intervention. As with our study, they found change in weight to be significantly associated with change in HOMA-IR, and that HOMA-IR was adversely affected by weight regain. Authors demonstrated that the magnitude of improvement in metabolic measures was most pronounced during the weight loss phase, rather than regain phase. Other studies[72,227] also show similar results. It may be that changes in insulin and HOMA-IR are more sensitive during a period of weight loss but not during weight regain. Or, this may

point to variability in factors associated with weight regain, such as behavioral, environmental, genetic, clinical, and molecular factors[228].

There are some limitations to our study that need to be addressed. First, we do not have any control groups with which to compare our results. Second, although we found significant associations between changes in weight and changes in metabolic measures, we cannot make any causal inference. We also used HOMA-IR as a measure for IR, and we acknowledge that there are caveats when using quantitative measures of IR. Nevertheless, HOMA-IR has been shown to be a good measure in the early detection of the development of type 2 diabetes[229], and has been well-validated when compared to the hyperinsulinemic euglycemic clamp, and is particularly effective in non-diabetic, insulin resistant populations[230]. Finally, because we included healthy individuals within specific ages and BMIs, our results cannot be generalized to populations outside of our age and BMI ranges or populations with chronic health issues. Our study also has several strengths. First, we have a unique population of individuals who lost and regained ≥10 lbs. We also incorporated a longitudinal study design with data collected at five time points over the course of two years. This is in contrast to the majority of studies that are either cross-sectional or collected data at only one or two follow-up points. We had a sample that was diverse by race and gender.

In conclusion, moderate weight loss was associated with significant improvements in metabolic measures. During weight regain, improvements in metabolic measures were not sustained. Strategies are needed to circumvent weight regain in order to maintain a healthy metabolic profile, preventing diseases such as type 2 diabetes and cardiovascular disease.

Table 4-1 Baseline sociodemographic and clinical characteristics of the participants

Characteristic	Men (n=13)	Women (n=53)	Total (N=66)
Age (years)	51.5 (7.2)	47.6 (7.3)	48.4 (7.4)
BMI (kg/m^2)	35.4 (4.6)	34.3 (4.3)	34.5 (4.4)
Education (years)	16.2 (3.7)	16.1 (3.1)	16.1 (3.2)
Weight (kg)*	111.9 (14.3)	92.8 (13.7)	96.5 (15.7)
Waist Circumference (cm) *	119.3 (11.4)	105.1 (12.3)	107.9 (13.3)
Body Composition (%)*	33.9 (5.3)	44.0 (4.5)	42.0 (6.1)
Insulin (μU/mL) *	21.6 (8.1)	15.8 (4.9)	16.9 (6.0)
Glucose (mg/dL) *	101.7 (6.1)	95.5 (9.0)	96.6 (8.8)
HOMA-IR*	5.4 (2.1)	3.8 (1.5)	4.1 (1.7)

Mean (SD)
*p<.05 between groups (t-test results)

Table 4-2 Percent change in weight and metabolic measures from baseline to 6, 12, 18, and 24 months (N=66)[1]

Characteristic	6 months	12 months	18 months	24 months
	Mean (SE^2)	Mean (SE^2)	Mean (SE^2)	Mean (SE^2)
Weight	-10.7 (0.6)*	-9.6 (0.9)*	-5.1 (0.9)*	-3.3 (0.8)*
Insulin	-8.9 (2.9)*	-6.3 (3.1)*	-1.2 (3.2)	4.9 (4.3)
HOMA-IR	-9.6 (3.3)*	-8.2 (3.7)*	0.6 (3.9)	8.4 (5.5)

[1] Results are least squares means from linear mixed models with time effect
[2]standard error
*significant difference from baseline (p<.05; p-values from F tests)

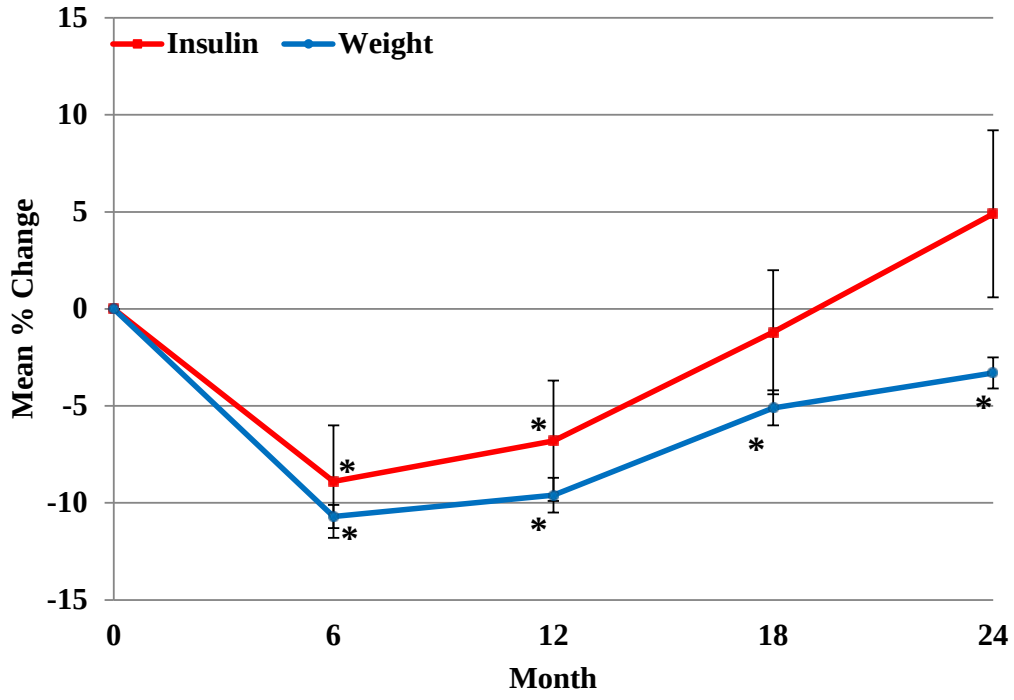

Figure 4-1 Percent changes in weight and insulin over time (N=66)

*p<.05 at respective time points

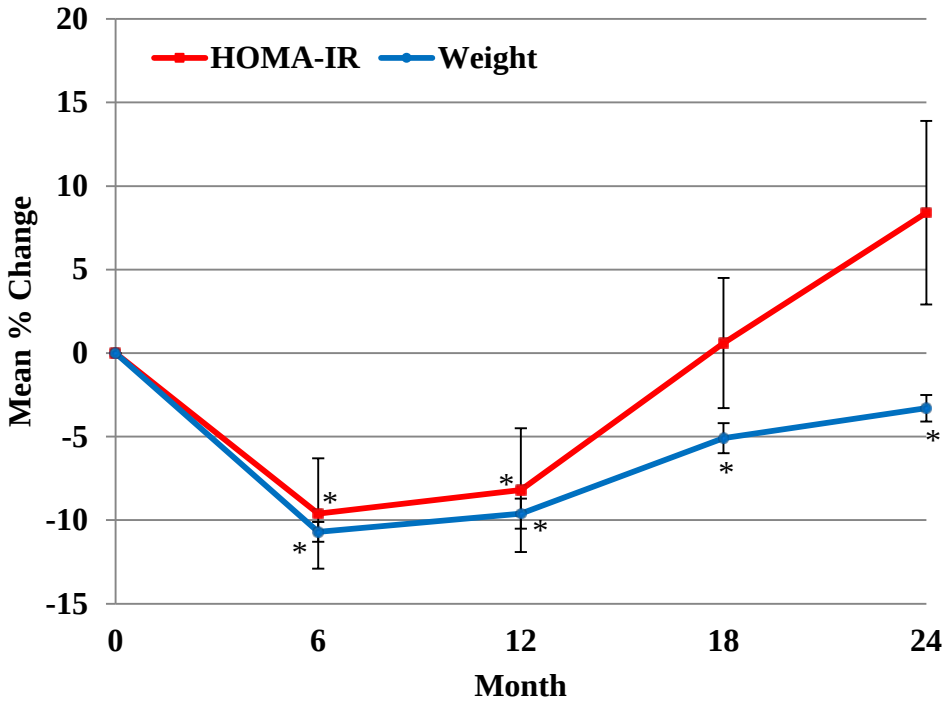

Figure 4-2 Percent changes in weight and HOMA-IR over time (N=66)

*p<.05 at respective time points

Table 4-3 Linear mixed modeling of percent changes in metabolic measures over time for every percent change in weight[1]

Characteristic[2]	β	SE[3]	P[4]
Insulin	0.5	0.1	<.0001
HOMA-IR	0.8	0.2	<.0001

[1]Adjusted for time, age, race, gender, baseline weight, baseline energy intake, percent change in energy intake, and baseline log-transformed insulin or HOMA-IR
[2]Log-transformed
[3]standard error
[4]P-values are from F-tests

5.0 THE ASSOCIATIONS BETWEEN WEIGHT CHANGE, CYTOKINE POLYMORPHISMS, CIRCULATING CYTOKINE CONCENTRATION AND INSULIN RESISTANCE AMONG OVERWEIGHT/OBESE ADULTS

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5.1 ABSTRACT

Background: Abnormal cytokine secretion is partly responsible for obesity-associated complications such as insulin resistance (IR). Single-nucleotide polymorphisms (SNPs) in cytokine genes may be associated with IR. Whether SNPs influence changes in metabolic risk factors during weight loss treatment and weight regain is underexplored.

Objectives: To determine whether SNPs in genes encoding IL-6, TNF-α, adiponectin, and IL-10 are associated with changes in cytokine concentration and IR among obese adults who lost and regained ≥ 10 lbs.

Methods: Eight SNPs in four cytokine genes (*ADIPOQ*: rs1501299, rs266729; *IL-10*: rs1800896, rs1800871, rs1800872; *IL-6*: rs1800795; and *TNF-α*: rs1800629, rs1800630) were genotyped in 65 participants (mean BMI=34.6±4.3 kg/m2) from the SMART Trial, a 24-month clinical trial of weight loss treatment. IR was evaluated using the homeostatic model assessment (HOMA-IR). Multiple linear regression was used to examine the association between each SNP and baseline changes in cytokines and HOMA-IR, controlling for age, ethnicity, gender, baseline weight, and for HOMA-IR, baseline adiponectin and TNF-α. Linear mixed models examined the association between each SNP and percent change in cytokine and HOMA-IR from baseline. For SNPs, a dominant model was used. All SNPs were in Hardy-Weinberg equilibrium.

Results: Compared with individuals with the TT genotype, variant allele C carriers for rs1800896 had higher baseline HOMA-IR [b(se)=1.0 (0.4), p=0.02]. Compared with individuals

homozygous for the major allele, carriers of minor alleles A and T for rs1800871 and rs1800872, respectively, had lower baseline HOMA-IR [for both SNPs: b(se)=-1.2 (0.4), p=.01]. There were no significant associations between SNP and either percent change in cytokine concentration or percent change in HOMA-IR over time. No significant interactions between SNP and weight were observed for either baseline or percent change in outcomes.

Conclusion: SNPs in *IL-10* were associated with baseline HOMA-IR. SNPs did not modify the association between weight and obesity-associated outcomes. These preliminary findings support the notion of genotypic differences in IR for IL-10 gene SNPs, but not the association between SNPs and changes in obesity-associated complications resulting from a weight loss intervention. More research with a larger sample size is needed to confirm these findings.

5.2 INTRODUCTION

Obesity is considered a chronic, low-grade inflammatory disorder, characterized by an increase in pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α, and a decrease in anti-inflammatory cytokines such as adiponectin (AdipoQ) and IL-10. Insulin resistance (IR), a major contributor to the development of type 2 diabetes, is prominent in obesity, and adipose tissue IR contributes to the development of cardiovascular disease[69]. Cytokine levels can thus be considered mediators in the association between obesity and the development of IR. Weight loss has been shown to prevent cardiometabolic diseases such as IR and improve cytokine function, although results are not conclusive.

Several single-nucleotide polymorphisms (SNPs) in cytokine genes have been examined in relation to circulating cytokine concentration. SNPs in cytokine genes indicate a predisposition to and/or different clinical features or outcomes of IR[160]. Examples include SNPs in *IL-6* (rs1800795), *TNF-α* (rs1800629, rs1800630), *AdipoQ* (rs1501299, rs266729), and *IL-10* (rs1800896, rs1800871, rs1800872). Evidence from the literature has shown these SNPs to be associated with circulating cytokine concentration, but the results have been mixed. Some studies have shown a significant association between these variants and circulating cytokine concentration[26,32,34,181,184,193], while others have not[35,180,190,191]. Likewise, there are mixed results with respect to IR, with an association between cytokine gene SNPs and IR being found in some[34,170,183,184] but not all[171,180,188,202] studies.

Since obesity is associated with both cytokine expression and IR, it is possible that cytokine SNPs and weight interact to affect IR. Yet, few studies have investigated how genotype of cytokine SNPs affects IR in response to a weight loss intervention. Given the variability in cytokine levels and IR among genetic studies and the limited literature on the effect of genotype on IR after weight loss, the purpose of this study is to investigate the interrelationships between cytokine SNPs, weight change, and IR among overweight/obese adults enrolled in the SMART Trial, a 24-month behavioral weight loss intervention. The goal is to determine whether SNPs in *IL-6, TNF-α, ADIPOQ,* and *IL-10* genes influence expression of these cytokines and IR during weight loss and regain.

5.3 METHODS

5.3.1 Study Design

The current study was an ancillary study (K24-NR010742) in which genetic testing of candidate cytokine genes were conducted among a subset of participants from the SMART Trial (NCT00277771), a single-centered, 24-month clinical trial of behavioral weight loss treatment that used a repeated measures design. The recruitment, screening, and enrollment details of the SMART Trial are published elsewhere[217].

5.3.2 Study Population

A total of 65 participants who lost and regained ≥10 lbs of body weight at any time during the 24-month study were included in the analysis. Individuals were eligible for inclusion in the parent study if they were 18-59 years of age or overweight/obese (BMI 27-43 kg/m^2), and were excluded if they were heavy (≥4 drinks/day) drinkers; had an eating disorder; or had a current serious illness or unstable condition, including a fasting glucose level above 125 mg/dL at baseline. All participants provided written informed consent, and the study protocol was approved by the University of Pittsburgh Institutional Review Board.

5.3.3 Measures

Body weight and height were measured and a blood sample obtained for assessment of total adiponectin, IL-10, IL-6, and TNF-α at baseline, 6, 12, 18, and 24 months. Completion of a baseline medical and weight history form provided data for history of an inflammatory illness or weight cycling. Sociodemographic data were collected by questionnaire. Energy intake was measured in kilocalories per day (kcal/day) by two unannounced 24-hour dietary recalls, conducted on one leisure day and one work day.

5.3.3.1 Anthropometric measures

Height was measured with a wall-mounted stadiometer and recorded in centimeters. Weight and percent body fat were measured following an overnight fast using a Tanita Scale and Body Fat Analyzer (Tanita Corporation of America, Inc., Arlington Heights, IL) while participants wore light clothing and stood erect with bare feet on the scale's footpads.

5.3.3.2 Cytokine measures

TNF-α was measured by Luminex technology multiplex ELISA (Linco Research, Inc.; St. Charles, MO). The minimum detectable level (MDL) was 0.14 pg/mL, with an assay range of 0.64-10,000 pg/mL. The intra- and inter-assay CVs were 1.4-7.9% and <21%, respectively. A quantitative sandwich enzyme immunoassay technique was used to measure IL-6 (Quantiglo Human IL-6 Immunoassay; R&D Systems, Minneapolis), with the MDL ranging from 0.05-0.35 pg/mL and an assay range of 0.48-1500 pg/mL. The intra- and inter-assay CVs were 3.0-5.8% and 6.3-9.6%, respectively.

IL-10 was measured by quantitative sandwich enzyme immunoassay technique (Quantikine HS Human IL-10 Immunoassay; R&D Systems, Minneapolis). The MDL was <0.5 pg/mL, with an assay range of 0-50 pg/mL. The intra- and inter-assay CVs were 6.6-8.5% and 8.1-15.6%, respectively. Finally, the ELISA technique was used for the adiponectin assay (Quantikine Human Adiponectin/Acrp30 Immunoassay; R&D Systems; Minneapolis), with a MDL of 0.25 ng/mL and intra- and inter-assay CVs of 2.5-4.7% and 5.8-6.9%, respectively All cytokine assays were performed at the Laboratory for Clinical Biochemistry Research at the University of Vermont.

5.3.3.3 Metabolic measures

Blood samples were obtained for assessment of insulin and glucose levels, and both metabolic measures were analyzed at the University of Pittsburgh Graduate School of Public Health's Chemistry and Nutrition Laboratory. Insulin was measured using a radioimmunoassay procedure developed by Linco Research, Inc. Glucose samples were quantitatively determined by an enzymatic method described by Bondar and Mead (1974). The coefficient of variation (CV) for insulin was 8.2 +/- 0.7 (170) % and 2.0% for glucose. The intra-assay CVs for insulin and glucose were 7.0% and 1.6%, respectively. Insulin resistance was assessed by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), calculated as [fasting insulin (μU/mL) x fasting glucose (mg/dL)] / 405.

5.3.3.4 Genotyping

DNA was retrieved from whole blood and was extracted by centrifuging the blood, removing the white cells, and extracting DNA from the white cells using a simple salting out

procedure. Genotypes were generated using TaqMan allele discrimination with the ABI Prism® 7000 Sequence Detection System and SDS software version 1.2.3 (Applied Biosystems Inc., Carlsbad, CA), using pre-developed and commercially available TaqMan SNP Genotyping assays (Applied Biosystems). Each assay plate contained a negative control, and each SNP was genotyped independently by two different individuals and genotype discrepancies were addressed by going back to the raw data or re-genotyping.

5.3.4 Data Analysis

Summary statistics for the total sample were reported as either a mean (SD) or frequency count (%). All weight, cytokine, and metabolic variables were assessed for normality, and all non-normal variables were log-transformed. Hardy-Weinberg equilibrium (HWE) was checked for all SNPs with a chi-square test ($p \geq .05$ means SNP was in HWE). The dominant model, where carriers of the minor allele were grouped together and compared with homozygous major allele carriers, was used due to small numbers.

Multiple linear regression was used to examine the association between each SNP and baseline cytokine concentration or HOMA-IR, adjusting for age, gender, race/ethnicity, and baseline weight. Models for HOMA-IR were additionally adjusted for baseline adiponectin and TNF-α, which were shown to be significantly associated with HOMA-IR. The interaction between baseline weight (as a continuous variable) and each SNP on baseline cytokine levels and HOMA-IR was explored; the interaction term was dropped from the model if it was not statistically significant. Percent change from baseline to 6, 12, 18 and 24 months was used in the

analyses of weight, cytokines, and metabolic measures. Percent change from the study entry time point was calculated as [(follow-up – baseline)/ baseline) x 100].

Linear mixed modeling with linear contrasts was applied to assess the effects of SNPs on changes in cytokines and HOMA-IR from baseline. Models included time, age, race, gender, baseline cytokine or HOMA-IR, baseline weight, percent weight change, and an interaction term for each SNP x percent weight change. The interaction term was also dropped from the model if it was not statistically significant. In each model, a random intercept was used for each participant, and an unstructured covariance structure was assumed. P-values from the F-tests were reported. In all analyses, the significance level was set at $p<0.05$ for two-sided hypothesis testing. Analyses of the data were conducted using SAS version 9.2 (SAS Institute, Cary, NC) and IBM SPSS Statistics version 21.0 (IBM Corporation, Armonk, NY).

5.4 RESULTS

Baseline demographic and clinical characteristics for participants are shown in Table 1. The sample was predominately female (80%), White (88%), and middle-aged (48.7±6.9 years). The mean BMI of the sample was 34.6±kg/m2 and mean body fat composition was 42.1±6.1%. Mean baseline glucose was 96.5±8.9 mg/dL and mean insulin was 16.9±6.0 μU/mL. Overall, there was an 11% decrease in weight, a 13.7% increase in adiponectin, an 8.6% decrease in insulin, and a 9.2% decrease in HOMA-IR at 6 months (ps<.05). By 24 months, only adiponectin concentration significantly decreased with nearly total weight regain. SNP characteristics, including the minor allele frequency (MAF) are presented in Table 2 for *IL-6*, *TNF-α*, *ADIPOQ*,

and *IL-10*. These genes were selected based on the role of these cytokines in obesity and obesity-associated health outcomes. Although 65 participants were genotyped for at least one SNP, not all participants were able to be genotyped for all eight SNPs; therefore, there was variability in the number of participants within each genotype group. All SNPs were in HWE.

Table 3 outlines the association between each SNP and cytokine concentration, controlling for age, ethnicity, gender, and body weight. Overall, genotype was not significantly associated with baseline cytokine concentration. The association between genotype and HOMA-IR, controlling for age, ethnicity, gender, baseline body weight, baseline adiponectin, and baseline TNF-α are outlined in Table 4. Overall, *IL-10* SNPs rs1800896, rs1800871, and rs1800872 were significantly associated with HOMA-IR (ps<.05). Compared to individuals with the TT genotype, variant C allele carriers of rs1800896 had higher HOMA-IR [b(se)=1.0 (0.4), p=.02]. Compared with individuals homozygous for the major allele, minor allele carriers for rs1800871 and rs1800872, respectively, had lower HOMA-IR [for both SNPs: b(se)=-1.2 (0.4), p=.01]. Results of the linear mixed models, adjusting for covariates, are outlined in Tables 5 and 6. Overall, there were no significant associations between genotype and outcomes over time. There was, however, a significant within-group difference in percent change in HOMA-IR for rs1800872. Individuals with the GG genotype significantly decreased HOMA-IR by 6.0% (p=.02), whereas T allele carriers did not (-1.0%, p=.31).

5.5 DISCUSSION

These preliminary results reveal, in a sample of obese adults who lost and regained at least 10 lbs as part of a behavioral weight loss study, significant associations between SNPs in the *IL-10* gene and baseline HOMA-IR. However, we did not observe any significant associations between genotype and baseline cytokine concentration, or genotype and percent changes in cytokines or HOMA-IR over time.

At baseline, we observed minor allele carriers of *IL-10* rs1800896 to have significantly higher HOMA-IR compared to individuals with the TT genotype. We also found carriers of the variant A and T alleles of *IL-10* rs1800871 and rs1800872, respectively, to have lower HOMA-IR, compared with individuals homozygous for the major allele. These *IL-10* SNPs have been researched in the context of diseases such as asthma[231], gastric cancer[232], and Crohn's disease[201]. However, the association between these SNPs and obesity-associated complications is limited in the literature. To our knowledge, no studies have investigated the associations between rs1800871 and IR, and only one other study investigated the association between rs1800896, rs1800871 and IR. Scarpelli *et al.* examined the association between rs1800896, rs1800872 and HOMA-IR among non-diabetic obese Caucasian adults. They found that carriers of the minor allele had higher HOMA-IR compared to individuals homozygous for the major allele, but did not find an association between rs1800896 and HOMA-IR[34]. This is in contrast to our study where we found minor allele carriers of rs1800896 and rs1800872 to have higher and lower, respectively, HOMA-IR compared to carriers of the homozygous wild genotype.

It is difficult to explain why the results of the current study are in contrast to those of Scarpelli *et al.* The differences between the two studies could be due to differences in sample

size and ethnic origin of the populations. The global minor allele frequencies for the *IL-10* SNPs are different between racial/ethnic groups. Scarpelli *et al.* had a more homogeneous population, whereas our sample population was less homogeneous; we included eight individuals who were of non-Caucasian descent. However, when analyzing the data with Caucasians only, the results were similar. Therefore, there could be three explanations for disparate findings. First, these SNPs have not been well-researched in the context of IR; therefore, the alleles for rs1800896 and rs1800872 that would be considered the risk allele for IR is inconclusive. Second, Caucasians in the U.S. may not represent a homogeneous population; varying ancestry within U.S. Caucasian populations may explain our results. Lastly, contrasting results may simply be due to different sample sizes. The current study included 65 individuals, whereas Scarpelli *et al.* included over 1100 non-diabetic obese individuals. Therefore, more clarification on the association between IL-10 expression and obesity-associated health outcomes is needed, in addition to more research with larger sample sizes on the role of *IL-10* SNPs in obesity and metabolic diseases.

We did not observe significant associations between SNP and percent change in cytokines or HOMA-IR over time. The associations between weight change, cytokine gene SNP, and IR is understudied in the literature. Curti *et al.* investigated the association between cytokine gene SNPs and cardiometabolic risk factors such as IR among obese Brazilian adults who participated in a lifestyle intervention, emphasizing a healthy diet, increased physical activity, and psychosocial stress management[40]. They found improvements in cytokines and insulin concentration (IR was not assessed) after the intervention in both genotype groups for each SNP. However, they did not investigate the association between *IL-10* SNPs and outcomes, nor did they explore the interaction between intervention changes (e.g. weight change) and SNPs on certain outcomes. They also only adjusted for age and gender, and may have biased results due to

lack of adjustment for other variables associated with their outcomes, such as baseline weight. Lastly, as with our study, they had a small sample size. Studies incorporating larger sample sizes are needed to confirm these findings.

Although we did not find any significant between-group differences in percent change in outcomes over time, we did observe significant within-group differences in HOMA-IR from baseline to 24 months for individuals with the GG genotype for rs1800872. Those with the GG genotype had significantly higher baseline HOMA-IR compared to T allele carriers; however, unlike T allele carriers, they had a significant decrease in HOMA-IR over time. This suggests that individuals with the GG genotype or individuals with higher baseline HOMA-IR may have greater improvement in metabolic outcomes following a weight loss intervention. Additional research is needed to determine differences in metabolic responses to weight loss.

There are some limitations to this study. First, we had a small sample size, thus giving a low power to detect associations between genotype and outcomes. However, these data are exploratory, and we believe that the findings are informative and may pave the way for the analysis of these associations in larger studies. Second, we cannot rule out the possibility of linkage disequilibrium between SNPs in the current study and SNPs that have yet to be discovered. Strengths of the study are that we examined SNPs in genes known to be associated with obesity and IR, and that there is a lack of information on these SNPs in relation to IR among obese adults enrolled in a weight loss study. Our sample population was unique; we included individuals who lost and regained $\geq$ 10 lbs during the weight loss study and included measurements at multiple time points, thus allowing us to determine the association of cytokine SNPs and longitudinal changes in our outcomes. We also investigated multiple SNPs in cytokine genes to give a more comprehensive view of the association between these SNPs and outcomes.

Future studies need to investigate the role that weight loss and regain play on the association between cytokine SNPs and obesity-associated complications such as inflammation and IR. Given the lack of attention in the literature to IL-10 expression in obesity and IR, more research is needed to clarify the role of this anti-inflammatory cytokine in obesity-associated complications to obtain a better understanding of how *IL-10* SNPs are associated with health outcomes. Future research should also include the investigation of haplotypes, which can provide more information on the association between genetic variants and disease. More research is also needed on the association between these SNPs and outcomes among individuals who are most at risk for obesity and IR, such as minority populations and individuals of a lower socioeconomic status. In addition, it is necessary to explore gene-gene interactions, and interactions between cytokine genes and environmental factors such as diet and physical activity.

In conclusion, SNPs in *IL-10* genes were significantly associated with IR. There were no associations between SNPs and changes in cytokines or IR over time. These preliminary findings suggest that there may be allelic differences in *IL-10* SNPs in terms of IR; however, more research with a larger sample is needed to confirm these results.

Table 5-1 Baseline demographic and clinical characteristics of the participants (N=65)

Characteristic	n (%)
Gender	
Female	52 (80.0)
Race	
White	57 (87.7)
	Mean (SD)
Age (years)	48.7 (6.9)
Weight (kg)	97.0 (15.4)
Waist circumference (cm)	
Female	105.4 (12.1)
Male	119.3 (11.4)
Body Fat Composition (%)	42.1 (6.1)
BMI (kg/m^2)	34.6 (4.3)
Adiponectin (µg/mL)	17.0 (9.2)
IL-6 (pg/mL)	2.6 (1.5)
IL-10 (pg/mL)	8.6 (4.0)
TNF-α (pg/mL)	5.1 (4.4)
Glucose (mg/dL)	96.5 (8.9)
Insulin (µU/mL)	16.9 (6.0)
HOMA-IR	4.1 (1.7)

sd, standard deviation; kg, kilograms; cm, centimeters; kg/m^2, kilograms/meters squared; µg/mL, micrograms/milliliter; pg/mL, pictograms/milliliter

Table 5-2 Description of cytokine polymorphisms

Chromosome (Position)	Gene	SNP	Major Allele	Minor Allele	MAF[a]	Global MAF[b]
3q27 (+216)	ADIPOQ	rs1501299	G	T	0.34	0.32
3q27 (-11377)	ADIPOQ	rs266729	C	G	0.16	0.29
7p21 (-174)	IL-6	rs1800795	G	C	0.31	0.50
1q31-32 (-1082)	IL-10	rs1800896	T	C	0.47	0.47
1q31-32 (-819)	IL-10	rs1800871	G	A	0.29	0.47
1q31-32 (-592)	IL-10	rs1800872	G	T	0.28	0.24
6p21 (-308)	TNF-α	rs1800629	G	A	0.13	0.17
6p21 (-863)	TNF-α	rs1800630	C	A	0.15	0.16

[a]Minor allele frequency
[b]MAF for global Caucasian population from the Single Nucleotide Polymorphism Database, National Center for Biotechnology Information/National Human Genome Research Institute

Table 5-3 Associations between SNPs and baseline cytokine concentration[a,b]

SNP	Gene	β[c]	SE[d]	P
rs1501299	ADIPOQ	-0.1	0.1	0.16
rs266729	ADIPOQ	-0.03	0.1	0.64
rs1800795	IL-6	-0.04	0.04	0.27
rs1800896	IL-10	-0.02	0.05	0.71
rs1800871	IL-10	-0.004	0.05	0.93
rs1800872	IL-10	0.001	0.05	0.98
rs1800629	TNF-α	-0.03	0.06	0.60
rs1800630	TNF-α	0.06	0.06	0.31

[a]Multiple linear regression models, adjusting for age, race/ethnicity, gender, and baseline weight
[b]Log-transformed cytokine concentration
[c]regression coefficient
[d]standard error
Referent group is carriers of the variant allele

Table 5-4 Associations between SNPs and baseline HOMA-IR

SNP/Gene	β[a]	SE[b]	P
rs1501299	0.03	0.03	0.38
rs266729	-0.02	0.03	0.62
rs1800795	-0.03	0.03	0.29
rs1800896	1.0	0.4	0.02
rs1800871	-1.2	0.4	0.01
rs1800872	-1.2	0.4	0.01
rs1800629	-0.02	0.03	0.60
rs1800630	0.03	0.03	0.38

[a]Regression coefficients, controlling for age, ethnicity, gender, baseline weight, baseline log-transformed adiponectin, baseline log-transformed TNF-α
[b]Standard error
Referent group is carriers of the variant allele

Table 5-5 Linear mixed modeling of associations between variants and percent change in cytokines over time[a,b,c]

SNP	Gene	β	SE	P
rs1501299	*ADIPOQ*	-5.5	5.2	0.34
rs266729	*ADIPOQ*	2.3	5.7	0.58
rs1800795	*IL-6*	2.4	7.1	0.83
rs1800896	*IL-10*	-1.0	9.3	0.96
rs1800871	*IL-10*	-2.1	9.0	0.98
rs1800872	*IL-10*	5.9	7.7	0.36
rs1800629	*TNF-α*	-2.2	8.7	0.64
rs1800630	*TNF-α*	1.2	8.7	0.49

[a]Models include time, age, ethnicity, gender, baseline weight, and respective baseline log-transformed cytokine concentration
[b]Cytokine corresponding to the gene
[c]Percent change in log-transformed cytokine

Table 5-6 Linear mixed modeling of associations between variants and percent change in HOMA-IR over time[a]

SNP	Gene	β	SE	P
rs1501299	*ADIPOQ*	-4.2	4.7	0.23
rs266729	*ADIPOQ*	4.8	5.0	0.42
rs1800795	*IL-6*	-3.7	5.0	0.37
rs1800896	*IL-10*	-2.0	5.4	0.59
rs1800871	*IL-10*	-6.7	5.1	0.22
rs1800872	*IL-10*	-5.0	5.1	0.36
rs1800629	*TNF-α*	-2.5	5.2	0.51
rs1800630	*TNF-α*	-0.3	5.5	0.82

[a]Models include time, age, ethnicity, gender, baseline weight, baseline log-transformed adiponectin, baseline log-transformed TNF-α

6.0 DISSERTATION SUMMARY

6.1 OVERALL SUMMARY

This dissertation examined the association between weight loss and regain, cytokine concentration, and insulin resistance among overweight/obese adults enrolled in the SMART Trial, a 24-month clinical trial of treatment for weight loss. In the first study, we found that moderate (11%) 6-month weight loss was associated with significant improvements in adiponectin and IL-6, but not IL-10 and TNF-α. We also found that, with nearly total weight regain, adiponectin was higher and IL-6 was lower than baseline, suggesting that moderate weight loss produced a lagged effect for these cytokines. Although 24-month values were better than baseline, there was some attenuation in cytokines at the end of the study. It may be that the benefits of weight loss on cytokines last for a certain amount of time, although this amount has not been determined in the literature. It is also possible that the amount of weight that is regained determines the course of cytokine expression. Nevertheless, from these results, it is clear that weight loss improves inflammation despite weight regain.

In the second study, we found that weight loss was associated with improvements in insulin concentration and HOMA-IR. However, despite our findings of a lagged effect for cytokines during a period of weight regain, we found that insulin concentration and HOMA-IR at

24 months surpassed baseline values. The literature has shown that insulin sensitivity that occurs after intentional weight loss is a predictor of subsequent weight regain, and that degree of weight regain after weight loss is dependent upon changes in insulin sensitivity as well as adherence to fat, calorie, and exercise goals from the weight loss intervention[233]. The increased insulin and HOMA-IR at 24 months beyond baseline values may reflect a decline in adherence to fat and calorie goals. Although we did observe higher levels of insulin and HOMA-IR at 24 months compared to baseline, these results were not statistically significant. It may be that improvement in metabolic measures was most noticeable during the period of weight loss rather than regain, suggesting that weight loss may be more important than regain in affecting insulin and HOMA-IR. There may be variability among individuals in insulin response to changes in body weight, especially among individuals of normal glucose tolerance[234]. It also may be that there is more variability in insulin response to regain compared to the weight loss period. Long-term changes in insulin action and secretion in weight regain is unclear.

The third paper was an ancillary study (K24-NR010742) in which genetic testing of candidate cytokine genes were conducted among participants from the SMART Trial. We examined the association between cytokine SNPs and cytokine concentration and insulin resistance. We found that SNPs in the *IL-10* gene were associated with baseline HOMA-IR. Specifically, the variant C allele for rs1800896 was associated with higher HOMA-IR compared to the TT genotype. We also found carriers of the variant alleles for rs1800871 and rs1800872 to have lower HOMA-IR compared to carriers of the homozygous wild genotype. We did not observe an association between other SNPs and cytokine levels or HOMA-IR, nor did we observe any associations between SNPs and percent changes in outcomes over time. However, we did find that individuals with the GG genotype for rs1800872 had a decrease in HOMA-IR

over time, despite having a higher HOMA-IR at baseline than T allele carriers. These findings suggest that genotype may play a role in IR. Also, although our results do not show any significant between-group differences in percent change in outcomes over time, we did observe a within-group difference in percent change in HOMA-IR over time for GG genotype carriers. This suggests improvement in IR during a weight loss intervention among individuals with the GG genotype. The literature is limited on the associations between cytokine gene SNPs and obesity-associated complications. There is much variability in these associations, with some studies finding cytokine gene SNPs to be associated with obesity-associated outcomes, whereas other studies have not. Only one study investigated *IL-10* SNPs in relation to metabolic outcomes[34], and these results were in direct contrast to our results. It may be that our sample size is too small to detect meaningful differences, or variability in results might be explained by differences in ethnic origin of the populations. It is also possible that, because this SNP has not been well-researched in the context of IR, the association between risk alleles for *IL-10* SNPs is inconclusive.

In summary, these three studies highlight the complex nature of obesity and weight change in relation to obesity-associated complications such as inflammation and metabolic outcomes. We revealed that weight loss was significantly associated with improvements in both cytokines and IR, but that these improvements were not sustained during a period of weight regain. This highlights the need for developing strategies to promote weight maintenance in the prevention of cardiometabolic diseases. We also highlighted the role of cytokine genes SNPs in IR, particularly in the *IL-10* gene.

6.2 LIMITATIONS

There are certain limitations that need to be addressed. We did not have a control group with which to compare our results. Although we found significant associations, we cannot make any causal inference. Male representation in the study was low; however, our sample was representative of those who typically seek treatment for weight loss. IR was measured by HOMA-IR, a surrogate marker derived from a mathematical model, rather than the hyperinsulinemic euglycemic clamp, which is considered the gold standard for measuring IR. However, HOMA-IR has been well-validated when compared with the gold standard. For the SNP analysis, we had a small number of individuals, thus giving a lower power to detect meaningful differences. There may also be issues with linkage disequilibrium between the SNPs used in this study and unknown SNPs. Finally, our sample population was healthy with specific ages and BMIs, thus limiting generalizability to other populations.

6.3 PUBLIC HEALTH SIGNIFICANCE

Obesity has reached epidemic proportions in the U.S. with over one-third of Americans being obese[1]. Concurrent with the growing obesity epidemic is the increase in type 2 diabetes and cardiovascular disease, both major causes of morbidity and mortality. The findings from this dissertation reveal that changing behaviors such as reducing energy intake and increasing energy expenditure, which lead to weight loss, can improve cardiometabolic outcomes and reduce inflammation. However, it is difficult to maintain weight, and weight regain can have a detrimental effect on one's health. Therefore, it

is necessary to devise strategies to prevent weight regain, thus reducing the risk of developing cardiometabolic diseases.

Obesity is a complex disorder, which includes the interaction between behavioral, environmental, and genetic factors. Cytokine gene polymorphisms can be informative in determining risk for IR and how an individual responds to a weight loss intervention. This dissertation revealed that individuals who were carriers of a particular allele had greater IR compared to individuals of the alternative allele. Tailoring weight loss interventions to specific genotypes may lead to an improved risk profile in those most susceptible to diabetes.

6.4 FUTURE RESEARCH

Future research needs to examine the associations between changes in adiposity and cardiometabolic risk factors such as inflammation and IR using advanced methods of adiposity detection, e.g. magnetic resonance imaging or computerized tomography. Visceral adipose tissue is known to be a greater risk factor for inflammation and cardiometabolic disease compared with subcutaneous adipose tissue[88], and advanced methods for measuring this depot may give insight into the association between adiposity and cardiometabolic risk factors. Subclinical measures of cardiovascular disease, such as pulse-wave velocity and coronary artery calcification, should be investigated in association with weight change, cytokine concentration, and cytokine gene polymorphisms. Future studies need to focus on increasing male participation in weight loss interventions. Findings from this dissertation revealed that males have higher insulin and adiponectin concentrations

at baseline compared with females, and a significant interaction between gender and percent change in weight on percent change in adiponectin over time revealed that males had a higher increase in adiponectin with weight loss compared with females. This suggests highlights a potential health benefit of weight loss, particularly for males, who may be at an increased risk of developing cardiometabolic diseases.

Inflammation is a hallmark of obesity and is implicated in the development of cardiometabolic diseases such as IR. Anti-inflammatory markers such as adiponectin have been well-researched in the literature in relation to obesity and IR. However, other anti-inflammatory markers such as IL-10 are very informative, but not well-researched. Therefore, there needs to be a greater understanding of the role of anti-inflammatory cytokines beyond adiponectin on health outcomes, and the effects of weight loss and regain on expression of these cytokines.

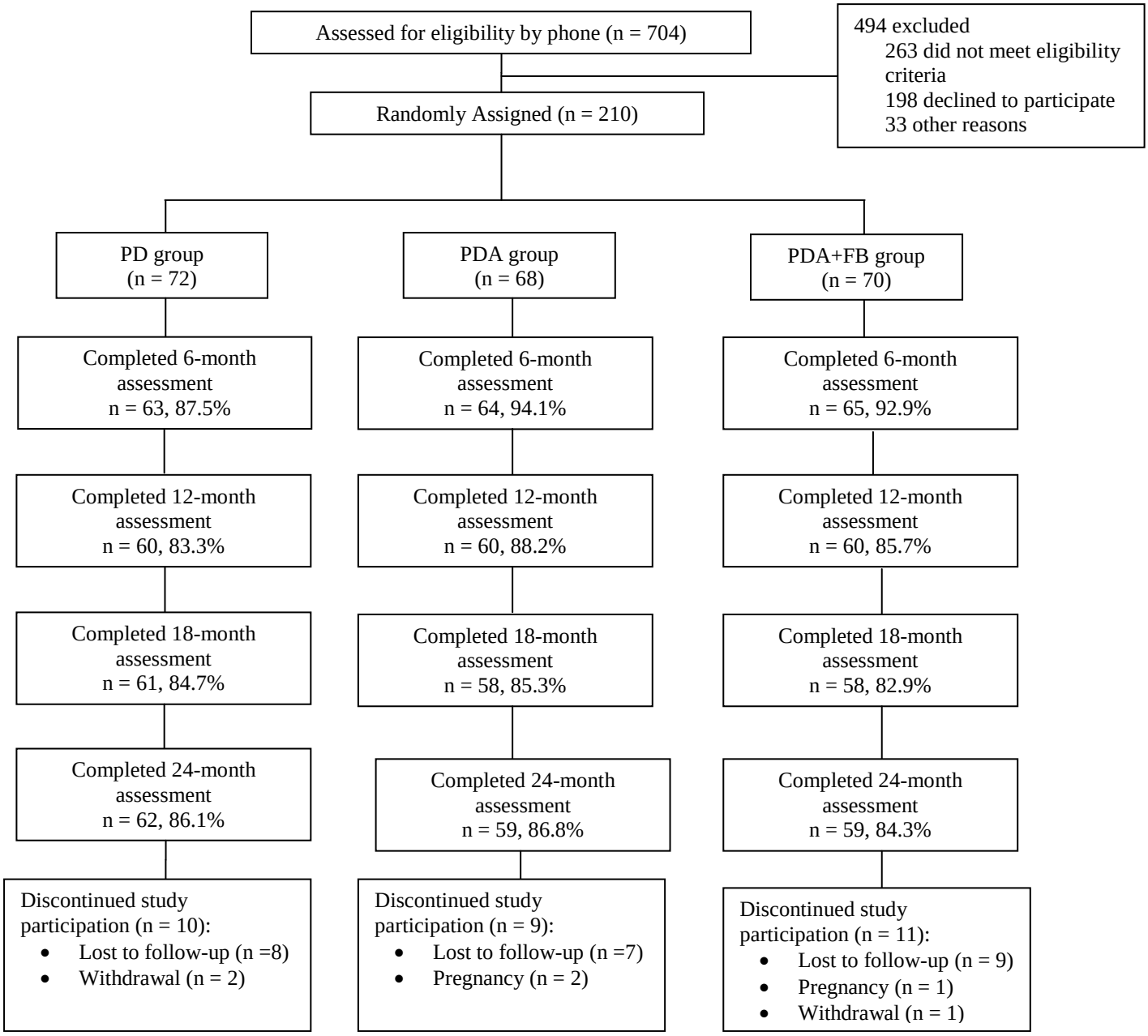
Although the SMART Trial was a 24-month clinical trial, and most weight loss studies are short-term (6 months or less), studies with longer follow-up are needed to determine the effects of weight regain on cytokines and IR. It is necessary to examine differences between individuals who lost and regained weight, those who lost and maintained weight, individuals who maintained obesity, and normal weight individuals, to decipher the role of weight cycling on health outcomes. More studies are needed examining these associations among populations who are known to have higher obesity and cardiometabolic disease, such as African-Americans[235].

For the investigation of cytokine gene SNPs and IR, future research should include larger sample sizes with enough power to detect differences. Because obesity is a disorder that is a combination of genetic, behavioral, and environmental factors, studies should explore gene-gene interactions, as well as gene-diet, gene-BMI, and gene-physical activity interactions. Haplotype analyses, which could potentially provide more relevant information, should also be conducted.

Studies should include genetic analysis among sociodemographic and racial/ethnic groups at risk for obesity-associated complications. This study included individuals of all racial/ethnic groups, although the majority of the participants were Caucasian. Race/ethnicity was controlled for in all models; however, this is not a very robust method for genetic analysis due to differences in allele frequencies between racial/ethnic groups. Therefore, the data were re-analyzed among Caucasians only, and the results were unchanged. This may hint at the lack of homogeneity among Caucasians in the U.S. A more robust method in genetic analysis to control for population substructure is the use of ancestry informative markers (AIMs). AIMs are markers not associated with disease that reflect a quantitative measurement of an individual's ancestry. Thus, controlling for AIMs in studies among non-homogeneous populations can be more accurate than controlling for self-reported race/ethnicity, and can account for variability in ancestry among individuals of the same race. The implementation of these methods to examine ancestral differences is critical in determining genetic associations among individuals with substantial admixture between continents[236].

APPENDIX

SMART TRIAL CONSORT DIAGRAM



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